

Planning Considerations Related to the Organic Contamination of Martian Samples and Implications for the Mars 2020 Rover

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1. Executive Summary

DATA GATHERED DURING RECENT NASA missions to Mars, particularly by the Rovers Spirit, Opportunity, and Curiosity, have provided important insights into the past history and habitability of the Red Planet. The Mars science community, via input through the National Research Council (NRC) Planetary Science Decadal Survey Committee, also identified the prime importance of a Mars sample return (MSR) mission to further exploration of the Red Planet. In response, the Mars 2020 Mission (Mars 2020) Science Definition Team (SDT) (Mustard *et al.*, 2013) was chartered by the NASA Mars Exploration Program to formulate a new rover mission that would take concrete steps toward an eventual sample return. The SDT recommended that the 2020 rover should select and cache scientifically compelling samples for possible return to Earth. They also noted that organic contamination of the samples was a significant and complex issue that should be independently investigated by a future committee. Accordingly, NASA chartered the Mars 2020 Organic Contamination Panel (OCP).

The OCP was charged with evaluating and recommending sample contamination requirements for the proposed Mars 2020. A further task was to assess implementation approaches in support of the investigation of broad scientific questions concerning the history and habitability of Mars.

Central to these objectives would be the ability to reliably differentiate indigenous martian organic molecules from terrestrial contamination in any future samples returned from Mars.

Early on during its deliberations, the OCP recognized that the scientific and planetary protection (PP) objectives of MSR are intimately linked, in that both rely heavily on measurements of organic molecules in the returned samples. In each case, a key aspect of the problem is being able to recognize and interpret organic molecules as indigenous to Mars against a potential background of Earth-sourced contamination. It was within this context that the OCP committee considered the structure for a set of measurement goals related to organic molecules in the returned samples that would be of common interest to science and PP.

The following is a summary of the most significant findings of the OCP regarding organic geochemical measurements that would be shared for both science and PP in relation to potential future MSR.

Rationale

- A key subset of both scientific and PP objectives could be met by a common set of organic geochemical measurements of returned samples. The science and PP teams would need to work as a fully integrated entity.

- Detection and characterization of indigenous organic compounds are of fundamental and critical importance in the search for ancient and extant life in martian samples.
- Because of the sensitivity of modern analytical instruments, we must accept that we would not be able to reduce *all* organic contaminants to non-detectable levels by *all* analytical techniques.
- Our ability to correctly interpret data from partially contaminated samples would depend on three major factors: (1) minimizing contamination at the start, (2) characterizing and understanding residual contamination throughout the mission, and (3) minimizing recontamination back on Earth.

Recommended approach

- Maintaining the original physical structure and geometry of the samples (*e.g.*, layering, gradients, grain boundaries, and cross-cutting relationships) and minimizing vibration and fracturing are critical to interpreting indigenous organic geochemistry since the spatial distribution patterns of molecules can be especially informative.
- A huge diversity of techniques for organic analysis exists as of 2014. More will be invented. Not all potential measurements would be possible on returned Mars samples given limited sample mass, nor would all be needed. Containment requirements may also limit access to some potential analytical methods. Accordingly, a two-step process, comprising initial survey measurements followed by more targeted analyses, is recommended. Effective early survey measurements in a future sample receiving facility (SRF) would be critical for establishing the full investigation plan.
- A small number of existing analytical techniques would be sufficient to provide the survey information required. These measurements would then inform which targeted methods should be applied.
- Protecting a measurement does not require contamination to be below detection limits, only that the interference with the measurement is acceptably low, stable, and well known. Thus, a comprehensive program to characterize the composition, concentration, and variability of residual contamination in the sampling hardware and cache would be essential.

Contaminant levels of concern

- At the level of individual molecules, organic compounds from biotic versus abiotic sources can be difficult or impossible to distinguish. Detection of *any* assemblage of biomolecules would likely require further and detailed investigation to establish its relationship to life.
- Avoiding growth of terrestrial microbes in a sealed sample tube, prior to their receipt by sample analysts, is of utmost importance. A strategy to mitigate this should be identified.
- We propose the following limits for organic contamination of returned Mars geological samples by specific compounds: 1 ng/g for Tier-I compounds and 10 ng/g for Tier-II compounds. Tier-I compounds are a selection of those molecules that are both common to and abundant across all life as well as additional compounds that are

considered particularly problematic. Tier-II compounds comprise all other organic molecules.

- Much of the organic contamination that accumulates on the collected samples would be delivered from spacecraft surfaces by direct contact.
- In the hypothetical case of a system with sample contact surface area of 30 cm² and contaminated with 20 ng/cm² organic carbon, collected samples would have a theoretical maximum of 40 ng/g (*i.e.*, 40 ppb) organic contaminants. The actual contaminant concentration may be less than 40 ng/g, depending on transfer efficiency.
- In order to achieve contamination levels for sample contact surfaces lower than 20 ng/cm², a more effective strategy for avoiding recontamination after initial cleaning than that used by the Mars Science Laboratory (MSL) would need to be implemented.
- Cleaning spacecraft surfaces to levels of 10–20 ng/cm² has been achieved in prior missions. Significantly lower levels of cleanliness are technologically feasible, and advisable, but would require engineering solutions to limit recontamination and the maintenance of these levels.
- Methods used for assessing hardware surface contamination should be demonstrated to have a known, reproducible efficiency in detection of the target Tier-I compounds.
- We propose a threshold for total organic carbon (TOC) contamination of geological samples of 40 ng/g.
- Molecular measurements provide a proxy for estimating total cellular/microbial particulate contamination that is much more robust and universal than microbial cell culture and growth. This finding is based on the fact that the vast majority of microbes cannot be cultured using standard methods.

Implementation strategy

- The overall organic contamination control strategy should involve monitoring for Tier-I compounds, monitoring of TOC, and broadband screening for Tier-II compounds above 10 ng/g.
- Witness plates and blanks would need to make the round trip to Mars. Many witness plates should be collected during Mars 2020 construction and be archived for future reference.
- In order to track the introduction of contaminants, the sampling strategy would need active control over witness plate exposure during discrete mission phases.
- The return of *in situ* drilled procedural blanks would be an important part of recognizing contamination and protecting the science of this mission.
- Samples of organic and biological material associated with the process of building the Mars 2020 spacecraft should be collected and preserved in a dedicated contamination archive facility. These samples should be available for analysis during and after mission operations.
- Baking all sampling and cache hardware in the presence of oxygen, followed by rapid isolation from contact with air, potentially provides a means to achieve orders-of-magnitude lower levels of organic contamination. We suggest that the project substantively investigate this possibility while evaluating sample hardware design options.

- Since we don't know the concentration of the organic molecules of interest in the martian samples, there is an unquantifiable scientific risk relating to detectability above background. The cleaner (or dirtier) the samples are, the more (or less) compounds we would be able to measure, and the more (or less) confident we would be in interpreting their origin. Scientific return versus sample cleanliness is a continuous function that is hard to cast in the terminology of required/not required, or success/failure.

2. Introduction

2.1. Introduction to the Mars 2020 OCP

The scientific successes of recent NASA Mars missions, including the rovers Spirit, Opportunity, and Curiosity, have provided important insights into our understanding of the past history and habitability of the Red Planet (*e.g.*, Fairén *et al.*, 2010; Hurowitz *et al.*, 2010; Ehlmann *et al.*, 2011; Grotzinger *et al.*, 2014; see also the 500 abstracts recently submitted to the 2014 8th International Mars Conference). These research findings include evidence of liquid water at the ancient planetary surface and the presence of environments that would be conducive to the existence of microbial life (Haskin *et al.*, 2005; Ehlmann *et al.*, 2011; Grotzinger *et al.*, 2014). The Mars science community, via input through the NRC Planetary Science Decadal Survey Committee, is emphatic that NASA scientific missions in the upcoming decade include an MSR mission to support a broad array of scientific and other goals (see NRC, 2007, 2011; MEPAG ND-SAG, 2008; Carr *et al.*, 2012; McLennan *et al.*, 2012). Returned martian samples could profoundly change our understanding of the evolution of Mars and the distribution of life in the universe. Consequently, despite the technical challenges of returning samples, the NRC gave a Mars sample-collecting rover its highest priority within the flagship class.

Subsequently, the Mars 2020 SDT was chartered by the Mars Exploration Program to define a Mars caching rover mission that would make significant and concrete steps toward possible return of samples from Mars (NRC, 2011). They recommended that the 2020 rover should carefully select and cache samples for potential future return to Earth, and noted that in order for the samples to be worth returning, they should be scientifically compelling (Mustard *et al.*, 2013). The SDT also noted that organic contamination of the samples was a significant and complex issue that should be investigated by a future committee. Accordingly, NASA has chartered the Mars 2020 OCP. The OCP comprised 12 members with expertise in astrobiology, PP, organic chemistry and geochemistry, analytical chemistry, and contamination control/containment engineering. *Ex officio* members from the Mars 2020 Project Team, the NASA Office of Planetary Protection, and the Mars Exploration Program Office also provided input for the committee's research.

Within this report, the term "organic geochemistry" is sometimes used, and this may not be familiar to all readers. We use this to refer to the study of organic molecules in geological samples, such as in rocks, soils, and meteorites. Organic geochemistry is a component of the broader field of "astrobiology," which also encompasses other kinds of investigations.

2.1.1. Mars and the potential for habitability. Several lines of evidence indicate that Mars once had surficial liquid

water (see, for example, multiple abstracts presented on this subject at the July 2014 8th International Mars Conference), but that this water disappeared from the martian surface long ago (Clark *et al.*, 2005; Goetz *et al.*, 2005; Haskin *et al.*, 2005; Fairén *et al.*, 2010; Hurowitz *et al.*, 2010; Leshin *et al.*, 2013). However, molecular evidence of past habitability may be provided by the presence of organic biosignatures or spatially defined geochemistry, protected for eons within rocks, as it has for pre-Cambrian life on Earth (Marshall *et al.*, 2007; Oehler *et al.*, 2009; Summons *et al.*, 2011; Bontognali *et al.*, 2012; Williford *et al.*, 2013; Briggs and Summons, 2014). The ability to collect and return samples of unoxidized sedimentary rocks, and to carry out sensitive, accurate, and precise organic geochemical measurements on these samples, would therefore provide one of the strongest lines of evidence as to whether Mars could, or ever did, support life.

Of relevance to considering the possibility and fate of martian organic molecules is that Mars is bathed in strong ultraviolet (UV) light during the day. The wavelength of UV radiation on Mars extends from approximately 190 to 400 nm, encompassing UV-C, -B, and -A wavelengths. Given that the martian atmosphere is thin, CO₂-rich, and ozone-poor, the UV reaching the surface of Mars has an approximately 1,000-fold greater biocidal effect than on Earth (see Beaty *et al.*, 2006; Hassler *et al.*, 2014; Rummel *et al.*, 2014). This radiation is believed to be responsible for the accumulation of oxidants, such as salts of perchlorate (ClO₄⁻), within the martian regolith (see Cockell *et al.*, 2000; Hecht *et al.*, 2009). This radiation can be effectively blocked by relatively thin layers (on the order of less than 1 mm) of UV-opaque materials such as dust or regolith, but the effect of the surface chemistry produced on organic molecules can be profound.

In 2012–2013, direct measurements of the flux of ionizing radiation on the surface of Mars were made for the first time using the Radiation Assessment Detector (RAD) carried on the MSL mission (Hassler *et al.*, 2014). During a 300-sol period, the RAD instrument detected a relatively constant ionizing radiation flux of approximately 0.180–0.225 mGy/day, composed almost exclusively of galactic cosmic rays; a single solar particle event on Sol 242 was recorded as a transient spike to 0.26 mGy/day. Although this amount of radiation has only a negligible impact on the survival terrestrial microbes (even radiation-sensitive ones, like *Escherichia coli*) at a time scale of 500 years (Rummel *et al.*, 2014), it has a very significant impact on the potential for preservation of organic molecules near the martian surface on a time scale of 4 billion years (Kminek and Bada, 2006; Pavlov *et al.*, 2012, 2014).

2.1.2. Charge to the OCP. It is assumed that the Mars 2020 rover would assemble a returnable cache of martian samples that may be returned to Earth, contingent on future decision-making by NASA and other sectors of the U.S. Government (see Mustard *et al.*, 2013). The state of the samples, required to meet these potential returned sample science goals, would thus dictate the conditions for how the samples would be collected, encapsulated, and possibly returned to Earth for laboratory analysis.

One of the reasons why sample return is a high priority to the science community is because of the unique capabilities of Earth-based instrumentation and analysis (see NRC,

2011, and references therein). High among these are the abilities to undertake high-precision, spatially resolved chemical and isotopic analyses and to perform wet-chemical extraction, derivatization, and separation of organics. Both would allow the detection of specific analytes of interest with greatly increased sensitivity and specificity over robotic instrumentation (NRC, 2007, 2011; Blake *et al.*, 2012; Mahaffy *et al.*, 2012). This substantial improvement in measurement sensitivity is especially important to the general scientific goals of MSR, which require that any organic evidence of habitability (past or present) is both verifiable and supportable in terms of its provenance and origin; the extraordinary claim of life on Mars would require, as first noted by Marcelo Truzzi, and reiterated by Carl Sagan, “extraordinary proof” (Sagan *et al.*, 1980).

The OCP charter outlines a specific and logical approach for generating recommendations about allowable contamination levels as input to the eventual requirement-setting process. It is expected that these recommendations would flow from sample-based measurement objectives, as illustrated in Fig. 1. The OCP was chartered with four primary technical tasks (see Appendices 1 and 2 online at http://mepag.jpl.nasa.gov/reports/OCP_2014_final_report_docx.pdf). A key ground rule is that the charter asks for a survey of *existing* instruments capable of measuring organics in geological samples—the OCP did not put any effort into forecasting analytic capabilities of the future:

- Based on current knowledge and capabilities, construct a list of measurements (and associated instruments/methods) anticipated to be made on the returned samples in support of objectives related to martian organic geochemistry.
- Determine the types and quantities of Earth-sourced organic contaminants of greatest concern, if they were found on the samples. Also, specify a TOC constraint.
- Assess possible implementation approaches for recognizing and distinguishing Mars-sourced organic mole-

cules in the samples from Earth-sourced organic molecular contamination.

- Evaluate draft Mars 2020 sample organic contamination requirements and draft verification methodologies (to be provided by the Mars 2020 Project).

A note from OCP about its charter: OCP has been asked to develop quantitative recommendations in response to a question for which the answer is intrinsically unknowable. The quantity and character of terrestrial organic contamination that would not significantly interfere with future measurement objectives depend strongly on what is actually in the samples, and exactly how it is measured in the future, and both of these are indeterminate. Thus, it is more realistic to think about the limit for maximum allowable contamination of samples as having some uncertainty, rather than a single number. In order to help with NASA’s requirements-setting process, the Panel ultimately has proposed a quantitative definition of “how clean is clean enough,” but in doing so, we encountered significant differences of opinion within the Panel, and we had few criteria to evaluate these differences. In addition, although science and PP both have a vested interest in the organic cleanliness of returned samples, the OCP’s results are most concrete in the area of measuring and interpreting organic molecules in rocks/soils. The issues around PP’s needs to implement policy and to comply with broader requirements (some of which relate to risk issues) are in part non-technical, and the OCP was not provided with criteria to evaluate this.

By focusing on levels of contamination in returned samples, our deliberations subsume a number of related activities. The Mars 2020 sampling rover would not be the only event in the “lifetime” of these samples (Fig. 2). The problem is simplified somewhat by the facts that the process we are trying to minimize is *terrestrial* organic contamination and that the samples are assumed to be contained while on Mars and in transit (see, *e.g.*, McLennan *et al.*, 2012; Mustard *et al.*, 2013; Beaty *et al.*, 2014). To the extent

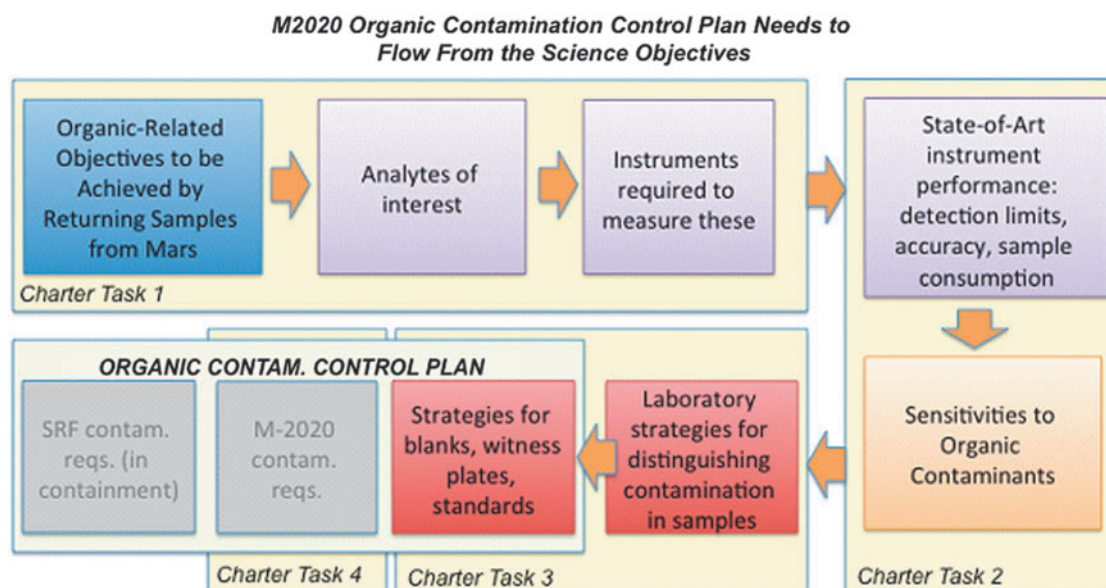


FIG. 1. Graphic representation of the logical flow of the 2014 OCP study. M2020 (M-2020), Mars 2020 Mission.

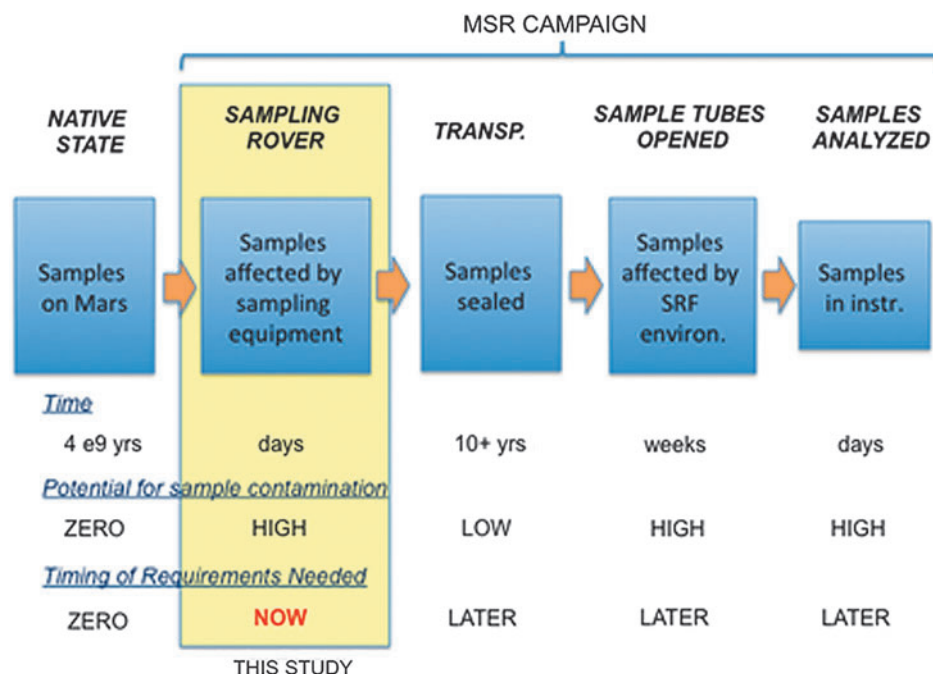


FIG. 2. Stages in the history of the samples throughout the notional MSR campaign. The yellow box illustrates the focus of this study, although a critical point is that levels set at this stage of the campaign would impact everybody downstream. Note that within the scale of the lifetime of the Mars 2020 rover, the samples are assumed to be vulnerable to organic contamination only until each sample is sealed, which would be a matter of days, not years.

that OCP discussed flight hardware, we focused on the baseline Mars 2020 rover, including sampling apparatus and caching system. However, organic contamination issues would eventually need to be considered for the entire lifetime of the samples, particularly in regard to design of the SRF. A second consideration is that of cross-contamination (of organics) between martian materials. Although of lesser concern for the scientific objective of detecting Mars-sourced organic compounds, it is nevertheless essential to the eventual conduct of scientific research. For the purpose of limiting the scope of this study, the OCP was asked to accept the assumption that cross-contamination (from one martian sample to another) on Mars would be insignificant.

2.1.3. Introduction to the proposed Mars 2020. The SDT (Mustard *et al.*, 2013) outlined three broad objectives for Mars 2020: (1) understand the local geology, (2) assemble a *returnable* cache of samples, and (3) make a contribution to preparing for the eventual human exploration of Mars. Of these, only (2) is relevant to the charge to this Panel. In this context, a “returnable” cache is one that could reasonably fulfill both scientific objectives for its study and PP needs for the protection of the public safety. Highly contaminated samples would presumably violate both requirements. In order to achieve these objectives, the proposed Mars 2020 rover would explore a site interpreted to have high astrobiology potential, conduct preliminary analyses using its onboard instrumentation, and carry sampling equipment needed to populate a cache with the most compelling samples.

As currently envisioned, the sample collecting and caching system would include a robotic arm with a turret-

mounted sample acquisition tool (“drill”), a cache canister with sample tubes and plugs (Fig. 3), bit boxes for coring, brushing and abrading bits, and a blank material for quality control (Mustard *et al.*, 2013). The SDT suggested that Mars 2020 have a minimum capability to collect 31 samples of 15–16 g each. Mustard *et al.* (2013) specifically called for the sealing of individual samples as quickly as possible after their acquisition. The purpose of this sealing is primarily to prevent the gain or loss of dust or volatile components, most importantly water and organic molecules. At the time of the writing of the SDT report (Mustard *et al.*, 2013), it was not clear how tight a seal was technically possible, and the SDT recommended a threshold requirement that the seals be “dust-tight,” with a baseline requirement that they be airtight (see their Finding 6-6). Clearly, from the point of view of the investigation of organic molecules in the samples, gas-tight seals on each sample would be a very high priority, so as to prevent their contamination after collection. If it is technically possible, we recommend that gas-tight sample sealing be a threshold-level requirement (with the quantification of the leak rate to be determined). Also, note that interpretation of potential eventual returned sample data may have less ambiguity if the sample tubes were also sealed during the voyage to Mars (this would eliminate some possible vectors for contamination), although the implementation planning is left for others.

Although Mars 2020 is a heritage-driven mission, the organic contamination considerations may depart significantly from heritage. For example, a central part of MSL’s strategy involved dilution of contaminants (aka “dilution cleaning”), and given the sampling concepts for Mars 2020, it may not be possible to use this approach to clean the

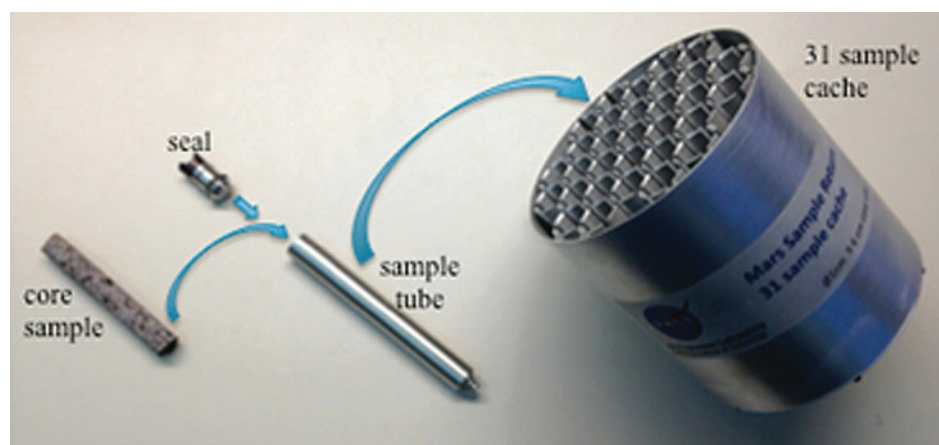


FIG. 3. One notional concept for the cache canister, sample tubes, and plugs to be carried on the proposed Mars 2020 rover (from Mustard *et al.*, 2013). Alternative hardware configurations are possible (*e.g.*, Zacny *et al.*, 2014). The essential aspect for the purpose of this report is the left half of this diagram, illustrating an encapsulated sample.

sampling chain. In addition, the instruments used to evaluate the quantity and character of contamination on the samples collected by Mars 2020 would be broader and more comprehensive than those used by MSL.

2.1.4. Definition of “organic contamination.” Although the meaning of “organic contamination” is generally well understood, there are several ambiguities that we must clarify. First, the term “organic” (as applied to molecules, or carbon) is itself ambiguous and is not strictly defined by the International Union of Pure and Applied Chemistry (IUPAC) (see Appendix 3 for definitions of terms). For example, some carbon-containing compounds are not reduced [carbon disulfide (CS_2)], others contain no C-H bonds [Teflon, $(\text{C}_2\text{F}_4)_n$, urea, $\text{CO}(\text{NH}_2)_2$], others are minerals (diamond, graphite), etc. Moreover, some compounds that are clearly inorganic have the potential to interfere with analyses of organic compounds, whether by confounding instrument readings (isobaric or spectral interference) or by binding, oxidizing, etc., organic compounds in the sample matrix (often referred to as “matrix effects”).

For the purposes of this Panel’s deliberations, we defined “organic contamination” as any substance that significantly interferes with our ability to detect the presence of martian organic compounds or prevents our confidently determining that an organic compound is of martian and not terrestrial origin.

We specifically did not include metal carbides within the scope of the above definition, because these were viewed as unlikely to cause significant problems (based on widespread terrestrial analytic experience). However, we are aware that background carbon contamination due to carbon-bearing stainless steel has been shown to contribute to contamination issues in some fluid inclusion and meteorite studies that use grinding and extraction approaches (Sherwood Lollar, personal communication, 2014), and that backgrounds have been successfully lowered by using low-C steel such as 316 stainless steel. If concern about the potential significance of metal carbide contamination on future organic measurements increases, we advise that this subject be revisited by a future group.

2.1.5. A note about units. For most of the measurements of organic molecules in geological samples (*e.g.*, rocks and soils), the data are typically reported as nanograms (or nanomoles) of molecules detected per gram of material analyzed (see Appendix 4). Such measurements would presumably be made on a split of the sample, which defines the denominator (and thus investigation of different sample splits can have legitimate differences between them). Other instruments listed in Appendix 4 make their observations using optical means. When applied to opaque objects, like metals, this gives a measurement of the surface. When such methods are applied to rocks, since most rock-forming minerals are transparent to optical investigations, such data respond to the sample surface plus the immediately underlying rock down to a depth of $10\ \mu\text{m}$ or more. The data from such investigations therefore commonly represent a combination of contamination molecules on the sample surface and indigenous molecules in the outermost volume of the sample. In order to systematize the discussion in this report, we have standardized the units to ng/g (which is equivalent to ppb by mass) and $\mu\text{g/g}$ (which is equivalent to ppm by mass).

2.2. Previous work on organic contamination control of acquired samples

Several previous groups have studied the question of permissible levels of organic contamination in returned samples in general as well as in past/ongoing sample-based investigations at Mars. Although our current thinking on this subject certainly benefits from recent geochemical measurements of martian materials (*e.g.*, Freissinet *et al.*, 2014) that were not available to earlier groups, it is instructive to understand how thinking on this subject has evolved through time:

Apollo (1969–1972). The first requirements for the organic cleanliness of spacecraft-acquired extraterrestrial samples originated in the Apollo Program (see recent summary by Calaway *et al.*, 2014). Although there was a desire to make samples available for measurement with organic contamination below $1\ \text{ng/g}$ (Flory *et al.*, 1969; Simoneit and Flory, 1971; Simoneit *et al.*, 1973), this was not technically

possible at the time (although this number is sometimes quoted in the post-Apollo literature). Prior to the flight of Apollo 11, organic contamination levels within the Lunar Receiving Laboratory (LRL) were observed as high as a sample equivalent of 1,000 ppm. Revamping laboratory sample handling and cleaning procedures reduced this level of organic contamination to < 1 ppm for the Apollo 11 samples (Flory and Simoneit, 1972; Simoneit *et al.*, 1973). After Apollo 11, Simoneit and Flory (1971) prepared a comprehensive analysis of potential pathways of surface contamination of Apollo samples, including contaminants arising from the LRL, Apollo lunar sample return container (ALSRC) and contents, Apollo lunar hand tools (ALHT), exhaust products from the Lunar Module (LM) [LM outgassing, venting of tanks, and Primary Life Support System (PLSS)], astronauts' suit leakage, astronauts' suit abrasion, and cleaning at the White Sands Test Facility (WSTF). Within the then ongoing Apollo program, this led to improved wet-chemical cleaning methods that led to an interpreted average of $10\text{--}100\text{ ng/cm}^2$ of flight hardware organic contamination for Apollo 12–15 [based on analysis of the ALSRC Aluminum York Mesh witness plates (Flory and Simoneit, 1972)]. Most Apollo LRL sample handling tools and containers were cleaned at WSTF to between 1 to 10 ng/cm^2 , although the state of their cleanliness at the time they were used to interact with samples is unknown. For Apollo 12, the Ottawa sand organic monitors showed some levels $< 1\text{ ng/cm}^2$ for the high vacuum complex in the LRL, and organic contamination levels on lunar samples were reduced to below $0.1\text{ }\mu\text{g/g}$. The level of detection for many organic geochemistry research laboratories at the time was about 1 ng/g (Calaway *et al.*, 2014). After Apollo 14, the Apollo missions did not mitigate against organic contamination. However, it is known that some types of sample containment added organics; for example, the SNAP line used for Apollo 14 and later missions, after abandoning the high vacuum complex, added as much as $10\text{ }\mu\text{g/g}$.

Viking (1976). The first missions to Mars to measure organic carbon in collected samples were the Viking missions. In terms of contamination control, the Viking missions were implemented with significant influences from the Apollo Program. The goal was to measure levels as low as $0.1\text{ }\mu\text{g/g}$ of total organics in small samples (100 mg) of collected soil, and a total contamination level of less than $1\text{ }\mu\text{g/g}$ (Flory *et al.*, 1974). However, the requirement for high-temperature “sterilization” of the entire lander system resulted in an extensive screening program for all materials, which could release potential contaminants by outgassing, as well as an inventory of materials flown and analysis of their compositions. A detailed process was developed for cleaning and processing the hardware (Flory *et al.*, 1974; Bionetics Corporation, 1990). The final cleaning of the sample path hardware in the organic analysis instrument [gas chromatography–mass spectrometry (GC-MS)] was done at the WSTF (see Seger and Gillespie, 1974): “The GCMS PDA and the collector head/shroud assemblies are sent to the NASA White Sands Test Facility (WSTF) to be organically cleaned to less than 1 ng/cm^2 with the procedures developed for the Apollo Moon Missions.” After sonic cleaning in triple-distilled ultrapure Freon solvent, the units were purged with low-pressure helium gas at high temperature (125°C) for 96 hr. Although there is evidence that during at least some

parts of the Apollo program metal surfaces were cleaned to $< 1\text{ ng/cm}^2$ of TOC, there is very little documentation for either Apollo or Viking regarding recontamination after cleaning. A second concern is that the cleanliness of the GC-MS instrument was assessed by solvent rinses followed by GC-MS analysis of the rinse fluid; thus they measured what could be removed from the surfaces, not what remained on them. It is uncertain whether a layer of adventitious carbon (AC) would be fully detected by this approach. Although a transfer coefficient was not quantified, the capability of the system to achieve its organic cleanliness goals was verified by using pyrolyzed soil (500°C) as a blank using prototype hardware. The Viking system apparently achieved significantly lower levels of contamination than the formal requirement, based on the assessment of the actual results from the GC-MS instrument during the mission, which measured residues of cleaning solvents at levels less than 1 to 50 ng/g [depending on the compound (Biemann *et al.*, 1976; Biemann and Bada, 2011)].

Mars Phoenix Mission (2007). The Phoenix mission utilized heritage hardware not originally intended for use in organic-sensitive analyses. As a result, no organic contamination requirements were levied on the project, and organic cleanliness was approached on a “best-effort” basis. Important surfaces, including those of the thermal and evolved gas analyzer (TEGA) instrument, were open to the ambient atmosphere; thus recontamination by AC was all but assured. These problems notwithstanding, analyses of the assembled spacecraft surfaces indicated typical TOC loadings of $40\text{--}600\text{ ng/cm}^2$. Much of this contamination, up to 85%, was a perfluorinated lubricant (“Bray-type oil”) used in the spacecraft. Remaining contaminants were largely phthalates and aliphatic alcohols, acids, and esters. Palmitic (hexadecanoic) acid was a substantial contaminant on all of the surfaces tested, but was not quantified (M. Anderson, unpublished data, 2006¹).

MSL (2011). Planning for contamination control requirements. The Organic Contamination Science Steering Group (OCSSG) was chartered in 2004 to specifically consider issues of organic contamination for the MSL mission in particular, and martian surface lander missions in general. Their report (Mahaffy *et al.*, 2004) considered a similar range of subjects as the OCP, but focused particularly on (1) identifying contaminants of most concern, (2) understanding methods of quantifying residual contamination, (3) possible mitigation strategies, and (4) use of controls and standards.

Regarding contaminants of concern, the OCSSG considered a broad array of compounds of potential scientific interest. These included all major biomolecules, aromatic and aliphatic hydrocarbons, nitrogen-, sulfur-, and oxygen-containing compounds, sulfonic and phosphonic acids, and others. They did not explicitly include any halogenated organic compounds, as the potential importance of these compounds was not appreciated at that time. Compounds rated as being of highest scientific interest (ranked H or VH) included aromatic and aliphatic hydrocarbons, carboxylic acids, amines and amino acids, purines and pyrimidines, carbonyl compounds, and alcohols (Mahaffy *et al.*, 2004).

¹JPL Analytical Chemistry lab report R196a, 12/28/06.

To derive proposed quantitative cleanliness levels (*i.e.*, bare minimum levels), the OCSSG relied on the calculations of Benner *et al.* (2000), who considered average rates of delivery of organics to Mars by meteorites, the depth of mixing of these organics into surface materials, and potential mechanisms and rates of oxidation. They concluded that “reduced organic groups such as aromatics or their oxidation products at mixing ratios of hundreds of parts per billion to hundreds of parts per million are expected.” These investigators noted that to define individual molecular species within these groups, and to definitively understand oxidation pathways, measurements down to ng/g levels might be necessary. They (Mahaffy *et al.*, 2004) thus concluded:

Keeping terrestrial contamination to below 1–10 parts per billion in Mars samples should allow significant scientific conclusions to be reached concerning the fate of organic material delivered by meteorites. The total molecular carbon contamination allowed could be substantially higher (for example, 40 ng/g) if the contamination by specific critical species or classes was maintained at dependably constant levels. Although extinct or extant life on Mars has the potential to leave signature organic material in either much higher abundance than the parts per billion levels discussed above, the OCSSG concluded that a definitive search for such signatures could be implemented on MSL by maintaining terrestrial contamination below levels of 1–10 ng/g for relevant biomarkers.

The OCSSG went on to propose specific limits for those relevant biomarkers and for total reduced (organic) carbon in their Table 1. They also provided example calculations translating those sample-based requirements into hardware surface requirements, with the caveat that example requirements “...may be modified as the fidelity of contamination migration models increases.” Requirements for levels of specific compound classes were initially adopted essentially verbatim by the Project based on these recommendations, but were later waived as not being implementable. Reasons for this determination included limitations upon existing contamination measurement capabilities within laboratories of NASA Goddard Space Flight Center (GSFC) and JPL and the lack of resources to obtain equipment and carry out analytical methods development within the original project development time frame.

Early on, the MSL contamination control engineers conducted a trial to demonstrate analysis capability. Various analytical methods [optical and Fourier transform infrared (FTIR) microscopy, diffuse reflectance infrared (IR) Fourier transform (DRIFT)/FTIR spectroscopy, and GC-MS] were used to assemble a comprehensive set of results against the compound classes identified in Table 1. Although successful, the logistics of performing this elaborate suite of analyses were quite time-consuming and were deemed impractical for routine cleanliness verification on hundreds of spacecraft assays. The MSL team concluded that DRIFT/FTIR spectroscopy was adequate to verify the cleanliness of spacecraft hardware—including the sample transfer chain—to the level required to assure the integrity of the Sample Analysis at Mars (SAM) science goals [GC-MS, pyrolysis-GC-MS, and direct analysis in real time–mass spectrometry (DART/MS) were also used as needed on a case-by-case basis]. Rather than attempting to modify the OCSSG-proposed limits into a set of implementable requirements,

MSL proceeded with a waiver against the requirements as written: the specific verifications of the subcompounds in Table 1 were waived, while the overall 40 ng/g of TOC requirement was retained. In the context of the overall validation and verification program, the verification approach implemented by MSL was deemed robust enough to constitute a low risk to the mission science goals, SAM instrument objectives, and hardware safety.

A significant contribution of the OCSSG effort was to conduct and report experiments designed to understand the efficiency of physical transfer of organic contaminants from spacecraft surfaces to a simulated martian regolith. They considered a variety of organic compounds, regolith analogs, and metal surfaces, over temperatures ranging from –40°C to 25°C. In general, they found that the most significant factor affecting transfer efficiency was the level of abrasion of the metal surfaces: strong abrasion of surfaces resulted in up to 60% transfer, moderate abrasion produced 1.3–7.6% transfer, and passive transfer without abrasion yielded levels of 0.1–1.3%.

Actual performance. The history of the state of organic contamination of MSL’s sample contact surfaces as a function of time is as follows:

1. Initial cleaning. MSL sample acquisition, processing, and handling (SA/SPAH) hardware surfaces were cleaned to an initial level of approximately ≤ 25 ng/cm² (average) as determined by FTIR analysis (Herrick *et al.*, 2002) of dichloromethane solvent rinses of witness coupons that accompanied individual piece-parts through the cleaning process. Surface residue was not quantified below 20 ng/cm² because the requirement was 100 ng/cm².
2. At last access before launch. Solvent swab assays (hexane swab sampling, followed by analysis of the swab) prior to shipment of the flight system to the launch site, and then again just prior to last access before fairing encapsulation, showed an average of 23 ng/cm² (range, approximately 20–40 ng/cm²) for inner (not exposed) surfaces of the sample transfer chain surfaces. FTIR analysis showed that the contamination was in the form of aliphatic hydrocarbons/esters (Blakkolb *et al.*, 2014). This contamination was also analyzed by the SAM Science Team at GSFC by ground-based pyrolysis GC-MS, but no organic components were detected (Eigenbrode *et al.*, 2013a).

TABLE 1. MAXIMUM AMOUNTS OF CONTAMINATION THAT CAN BE TRANSFERRED TO ORGANIC AND MOLECULAR ANALYSIS EXPERIMENTS, AS PROPOSED BY THE OCSSG (TABLE 2 IN MAHAFFY *ET AL.*, 2004)

Compound class	ng/g of sample
Benzene or aromatic hydrocarbons	8
Carbonyl and hydroxyl containing compounds	10
Amino acids	1
Amines, or amides	2
Non-aromatic hydrocarbons	8
DNA	1
Total reduced carbon	40

3. At Mars arrival. The MSL team constructed models of the contaminant redistribution from surrounding hardware to sample chain during flight (using standard aerospace industry contamination transport modeling methodology; see, for example, Fong and Lee, 1992). These models predicted exposed sample chain surfaces would pick up some additional contamination from the cruise environment; these surfaces were expected to have up to 60 ng/cm^2 after landing. For the inner (*i.e.*, not exposed) sample transfer chain surfaces, the model assumed no desorption of the initial “at launch” surface contamination (approximately $20\text{--}40 \text{ ng/cm}^2$) during flight, and the model predicted no additional deposition to closed sample chain surfaces.
 4. During surface operations. The total sample contact surface area for MSL is approximately $1,000 \text{ cm}^2$, which is relatively large. If all of these contaminants were transferred to a single sample, an effective average surface cleanliness level of approximately 0.4 ng/cm^2 at the time of sampling would be needed to achieve delivery of a sample with 40 ng/g bulk organic contamination. Expressed differently, the first sample would have had a contaminant load of $>600 \text{ ng/g}$ of TOC (calculated using plausible contaminant transfer coefficients). As discussed above, sample chain surface cleanliness levels were actually predicted to be $20\text{--}60 \text{ ng/cm}^2$ at the time of landing. To achieve the required level of contamination in samples delivered to the SAM instrument, the MSL sample delivery architecture was designed around what was referred to as “dilution cleaning.” This consisted of passing one or more “throwaway” samples through the sample transfer chain, prior to delivery of the first actual samples of interest.
 - Based on a deterministic contamination transfer model for the scoop sample path, four dilution cleaning passes were executed prior delivery of the first sample for analysis by SAM. The contaminant load in that fifth sample was predicted to be approximately 10 ng/g (equivalent to 0.005 nmol , as chlorohydrocarbon). This level of predicted contribution from the sample transfer chain is similar in magnitude to the SAM instrument internal blanks run for the Rocknest samples reported by Glavin *et al.* (2013). The sixth and subsequent samples would be expected to have even lower concentrations of Earth-sourced organic contaminants, as the system is progressively used.
 - In the case of the drill sample path, the sample transfer model had prescribed between three and four cleaning cycles to achieve a $\leq 40 \text{ ng/g}$ target level. However, the Science Team elected to accept and analyze the first sample (because the SAM instrument does much more with solid samples than just measure TOC). This sample (from the John Klein site) was predicted to have had a contaminant load of approximately 430 ng/g of TOC [equivalent to $<0.2 \text{ nmol}$ as chlorohydrocarbon (Blakkolb *et al.*, 2014)]. In that sample, the SAM instrument detected chloromethane (CH_3Cl) and dichloromethane (CH_2Cl_2) in a combined total amount of approximately 73 nmol [average of four portions analyzed (Ming *et al.*, 2014)], although it is indeterminate how much of this is natural signal and how much is Earth-sourced contamination. MSL’s second drill sample was taken at the Cumberland site. The contaminant transfer model predicts that it was delivered to SAM with $\leq 69 \text{ ng/g}$ of TOC ($<0.03 \text{ nmol}$ as chlorohydrocarbon). SAM analysis of this sample detected $<20 \text{ nmol}$ of chlorohydrocarbon (and again, this would be a mixture of martian signal and contamination).
 - MSL’s sample acquisition system can contribute Teflon particles to the samples it collects, because of normal wear and tear of a compliant seal within the drill bit assembly (Eigenbrode *et al.*, 2013b). Teflon was chosen for this seal specifically because perfluorocarbons, such as Teflon, are in general devoid of other contaminants, and they have the added advantage that they generate few peaks in the GC-MS (*i.e.*, the SAM instrument) analyses. Further, the peaks they do generate have distinctive masses and are not likely confused with compounds of Mars origin. Late in the project life cycle, ground testing of an engineering model of the drill mechanism raised the concern that elevated levels of Teflon particles in the samples presented a potential safety risk to the SAM instrument. Further testing to understand the risk and minimize it was performed. The quantity of this contamination was predicted to be between 100 and $10,000 \text{ ppb}$ ($0.05\text{--}5 \text{ nmol}$ as perfluoroethylene) of Teflon. Based on this information, the potential quantity of Teflon in the samples was judged not to constitute an unacceptable risk to the instrument. For the John Klein sample, SAM did detect trace quantities ($0.1\text{--}0.3 \text{ nmol}$) of perfluoroethylene, a known pyrolysis product of Teflon (Ming *et al.*, 2014).
- Since direct verification of in-sample contamination levels is not technically possible at Mars, the predicted values for contamination quoted above are necessarily model-based. The model used predicts contributions of the sample transfer chain only, and does not include additional contamination within the SAM instrument itself (the MSL Project had an internal allocation of 4 ng/g out of the total 40 ng/g requirement for SAM).
- Note that the SAM instrument had contamination issues during the first 2 years of the MSL mission as a result of leakage of the derivatization solvent within the SAM instrument (and these issues are continuing up to the time of writing). Although this is a contamination-related concern, it is the result of a mechanical anomaly that is unique to SAM, and it has no significance to Mars 2020. This is not discussed further in this report.
- Origins Spectral Interpretation Resource Identification Security—Regolith Explorer (OSIRIS-Rex) (launch planned in 2016).** A Level-1 requirement for OSIRIS-Rex (see Lauretta *et al.*, 2014) is to return $>60 \text{ g}$ of *pristine* asteroid regolith. During initial formulation (pre-Phase A), OSIRIS-REx adopted the OCSSG contamination limits, but it became clear during Phase A that these were not achievable on a New Frontiers budget. OSIRIS-REx formed a working group with representation from the science team, flight system, instruments, systems engineering, and project management to ensure a broad and balanced consensus. In

the development of contamination control requirements the first obstacle was to define pristine as having contamination levels from the addition of species that do not interfere with the scientific conclusions. It was determined that alteration via loss of material (*e.g.*, water), was beyond the scope of the mission. The OSIRIS-REx contamination control requirements for organic and inorganic species were drawn from lessons learned from Stardust (Sandford *et al.*, 2010), from which OSIRIS-REx derives significant heritage. In addition, to avoid complicated analytical testing, proxies for compounds and species of concern were identified based on carbonaceous chondrite abundances and Stardust performance. These were converted from nanograms per gram of sample into nanograms per square centimeter of collector [touch-and-go sample acquisition mechanism (TAGSAM)] surface area assuming 100% transfer efficiency (although a vibration test show ppm levels of amino acid transfer under dry conditions), and shown in Table 2. It is important to remember that the OSIRIS-REx target is expected to be orders of magnitude richer in organic compounds than found on Mars.

To implement these levels, specific testing for amino acids on sampling hardware via witness plates is used. Amino acid requirements led to a ban on nylon, rubber, and latex use on all parts of the spacecraft. The hydrazine is limited by spacecraft design and operations. The other species are controlled by converting these surface requirements to particle and film requirements for theoretical IEST-STD-CC1246D and fractions of A. This results in a requirement of 100 A/2 under reasonable conditions.

OSIRIS-REx has a witness plate plan that includes assembly, test, and launch operations (ATLO) activities, gas samples, curation, and a series of sapphire and aluminum witness plates on the flight hardware. The exposure of these witness plates is adjacent to the sample collector and timed by mission phase. The flight witnesses will be returned with the samples. Analyses of ground witnesses will be performed off the critical path and under the watch of the Sample Analysis Working Group lead. Ground and flight witnesses will be distributed under the same rules as the asteroid samples.

A materials archive of ≥ 1 g of components and polymers with a reasonable path to the samples is planned. Determining which materials to archive required a high-fidelity outgassing model of the spacecraft. To minimize the diversity of materials and extent of the archive, the diversity

of polymeric is minimized and complex polymers (*e.g.*, silicones) are only used by waiver when no viable substitute can be found. Since the curation of hydrazine under flight-like conditions is impractical, flight hydrazine is analyzed before launch so that hydrazine that is identical both isotopically and in trace components can be mixed and fired through an archived thruster and catalyst bed if required by future researchers.

ExoMars (launch planned in 2018). The ExoMars 2018 Rover is planned to be the first life detection mission to Mars to be undertaken since Viking (for details, see Vago *et al.*, 2013). Its plans are to employ certain contamination control-related practices that were common to Viking's design and integration, including:

- Design to protect the sensitive surfaces based on segregation (sealed sample path) and overpressure (for example, pre-launch pressurization of the canister containing the drill)
- Material control based on elimination, conditioning, isolation or characterization (pre-flight and use of blanks during operation)
- Use of hot gas purging

As of August 2014, the requirements for the maximum amount of terrestrial organic contamination per gram of martian samples to be used for life detection on the ExoMars 2018 rover are as follows (Kminek, written communication, 2014):

- Material from biological sources ≤ 50 ng/g
- Monomers of Kapton, Mylar, and polytetrafluoroethylene (PTFE) ≤ 500 ng/g
- Fluorinated technical lubricants ≤ 500 ng/g
- Any other organic compound ≤ 50 ng/g

This is the contamination level applied to the subsystems involved in the acquisition, delivery, and analysis of martian samples for life detection. The philosophy of structuring the list this way is to have a lower level for biological organics and a higher level for engineering sources that have previously been characterized with standard outgassing testing, specific GC-MS analysis [protocol developed with the Mars organic molecule analyzer (MOMA)], and samples sent and tested by the MOMA team.

The ExoMars Rover instrument contents and sample pathway are planned to be assembled in an aseptic, International Organization for Standardization (ISO) 3 environment with ISO airborne molecular contamination class-9 (AMC-9) cleaning. In order to have empirical data regarding assembly and testing contamination data, the build philosophy contains two models that are planned to be employed to measure contamination sources during assembly and environmental qualification testing. During integration, indirect verification are planned to take place using witness plates, and routine cleanliness checks would be conducted with gravimetric analysis. End-to-end testing of the flight model is planned to verify cleanliness levels prior to launch. The MOMA instrument on the ESA ExoMars rover mission is being built (by NASA GSFC) with a required level of surface cleanliness of 10 ng of non-volatile residue (NVR)/cm².

MSR (MSR SSG II). The MSR Science Steering Group II (MSR SSG II) was formed in 2004 and chartered with re-examining the MSR goals and mission concepts in light of

TABLE 2. SUMMARY OF OSIRIS-REx CONTAMINATION PROXIES

<i>Species</i>	<i>Indicator</i>	<i>TAGSAM surface limit (ng/cm²)</i>
Amino acids	Biological contaminant, special for astrobiology	180
Hydrazine	Reduces organics	180
C	Organics	1000
K	Lithophile	170
Ni	Siderophile	34,000
Sn, Hg	Industrial contaminant	0.53, 0.46
Nd	Lanthanide lithophile	1.5
Pb	Chalcophile, special for chronology	0.79

the recent (at the time) results of the Mars Exploration Rovers (Spirit and Opportunity). This group comprised four sub-panels tasked with studying a broad range of issues; one of those was forward organic contamination (MacPherson and the MSR-SSG II, 2005). As with the OCSSG, they began by compiling a list of potential “indigenous martian organic molecules” (Table 6 in MacPherson and the MSR-SSG II, 2005) that included most of those from the earlier report, plus humic and fulvic acids, complex macromolecules, phospholipids, N- and S-containing gases, and hydrocarbon gases. They also ranked compounds in terms of relative scientific interest, and here they departed significantly from the OCSSG report (Mahaffy *et al.*, 2004). The highest-rated compounds of interest (ranked VH) were C1–C4 hydrocarbon gases, saccharides, amino acids, porphyrins, glycerides and phospholipids, and nucleic acids. The fact that only amino acids were ranked of “very high” scientific interest by both the MSR SSG II and OCSSG panels emphasizes the inherent difficulty in prioritizing the most interesting molecular targets. The MSR SSG II further stated that, considering the reactivity and volatility of these species, strict temperature control of samples during their return is likely necessary. They proposed a limit of less than -5°C as being desirable, $<20^{\circ}\text{C}$ as highly recommended, and $<50^{\circ}\text{C}$ as essential.

To derive quantitative limits for contamination, this group first considered those that had been recently proposed by the OCSSG. They comment (MacPherson and the MSR-SSG II, 2005):

In light of the great variety of state of the art analytical techniques that could be applied to organics analysis of returned samples; it would be desirable to realize even lower thresholds in a returned sample than these proposed thresholds for *in situ* analysis. For example, thresholds of less than 10 ng/g in total organic contamination and ng/g or below in the proxy compound classes listed above could enable robust conclusions to be drawn regarding the origin and processing of indigenous Martian organics found in the ng/g or higher abundances.

Next, in an effort to set a lower bound on what might be required for analyses of Martian rocks, the group considered analogous organic-poor rocks on Earth, as follows. Oxidized red sandstones and mudstones (“redbeds”) commonly contain 0.10–0.01 weight percent TOC. Of this, typically less than 10 $\mu\text{g/g}$ is extractable bitumen, and sometimes as low as 1 $\mu\text{g/g}$. Major compound classes, such as aliphatic hydrocarbons, often represent just 1% of that total extract, or 10 ng/g. Individual biomarker molecules, for example, *n*-alkanes and hopanes, are themselves typically only 1–10% (or less) of aliphatic hydrocarbons, and so are present at 0.1–1.0 ng/g (MacPherson and the MSR-SSG II, 2005, pp 29–30). Inferring that similarly low levels might be present in returned martian samples, the MSR-SSG II proposed organic contamination thresholds that were lower than those of the OCSSG by a factor of 4 (*i.e.*, 10 ng/g of TOC), and as low as 0.25 ng/g amino acids. Critical to their evaluation and conclusions is the assumption that terrestrial TOC-poor rocks are representative of what we might find on—and return from—the surface of Mars. The validity of this assumption was not explicitly discussed by the SSG II report, and was questioned by several panelists in the OCP.

MSR (Mars 2020 SDT). The most recent group to take on the subject of organic contamination was the Mars 2020 SDT (Mustard *et al.*, 2013). This group provided a technical

analysis of the Mars 2020 objectives, and looked closely at the susceptibility of those objectives to terrestrial organic contamination. They concluded that Objective C (sample return) was very highly susceptible to organic contamination, and provided a high-level strategy for controlling and characterizing organic contaminants. With regard to specific contaminant thresholds, the SDT did not perform any further analysis of scientific requirements, and instead recapitulated the proposals of earlier studies:

The degree to which interpretation of analyses of martian samples would be compromised by the presence of organic contaminants in samples containing indigenous martian organic material is unknown. Thus, we do not know what level of cleanliness would be appropriate. Contamination should be kept as low as reasonably possible and within the guidelines proposed by the MEPAG OCSSG and the MSR SSG report. In these reports a total of 40 ng/g reduced organic compounds, with sub-allocations of 1–10 ng/g for specific compound classes was proposed by OCSSG (2004) (this spec was specifically intended for *in situ* investigations, including MSL). The MSR SSG (2005) proposed a total of 10 ng/g of reduced organic compounds, with sub-allocations for specific compound classes—proposed for at least some MSR samples. These figures are estimates only of contamination levels needed to achieve the science objectives. As discussed, different levels may be required to meet planetary protection requirements, and those levels would be specified by advisory groups specifically chartered for that purpose.

The SDT ultimately proposed adopting the lower of the two earlier limits (10 ng/g of TOC) as the baseline (*i.e.*, desired) requirement, and the higher of the two (40 ng/g of TOC) as the threshold (*i.e.*, bare minimum) requirement. Although the text of their report implies that they supported the inclusion of separate thresholds for individual classes of compounds, their finding (Finding 6-12) does not explicitly state this. (See Section 4.1.3 of this report for the hybrid resolution to this issue proposed by the OCP.)

Summary. In summary (Table 3), three conclusions can be drawn from the above history: (1) It is technically possible to clean spacecraft sample-contacting surfaces (on Earth, prior to launch) to TOC burdens as low as 1 ng/cm², or perhaps even lower. (2) Previously cleaned sampling surfaces become recontaminated up to the point they are used to interact with samples, such that the state of their cleanliness is significantly worse (and hard/impossible to measure) than at the time of original cleaning. (3) Samples have been collected and analyzed by prior missions, or are proposed by future missions, with Earth-sourced organic contamination as low as about 40–50 ng/g.

Finding #1: Cleaning spacecraft surfaces to levels of 10–20 ng/cm² has been achieved in prior missions (*e.g.*, MSL). Significantly lower levels of cleanliness are technologically feasible, but would require approaches to limit recontamination and maintain such levels.

2.3. Key concepts

2.3.1. Terrestrial microbial life forms (alive or dead) as sources of organic molecular contaminants. From a purely analytical perspective, organic matter in samples returned from Mars can be broadly classified into (1) viable cells and

TABLE 3. SUMMARY OF ORGANIC CLEANLINESS GOALS AND OUTCOMES FOR SEVERAL PAST AND CURRENT MISSIONS

Mission	In-sample contamination requirement	Detection limit	Hardware cleanliness target	Hardware cleanliness achieved	In-sample contamination achieved
Apollo (Simoneit <i>et al.</i> , 1973)	Estimated approximately low to sub- $\mu\text{g/g}$	Approximately ng/g	1 ng/cm ² (capability driven)	10–100 ng/cm ²	Approximately 1 $\mu\text{g/g}$ to $\geq 0.1 \mu\text{g/g}$
Viking (MAE) (Flory <i>et al.</i> , 1974; Biemann <i>et al.</i> , 1977; Bionetics Corp., 1990)	$\leq 1 \mu\text{g/g}$	$\leq 10 \text{ ng/g}$	1 ng/cm ² (capability driven)	Some sealed MAE hardware may have been at or below the lower end of the Apollo range.	Methyl chloride: $\sim 15 \text{ ng/g}$ Fluoroethers: 1–50 ng/g
PHX (Mahaffy <i>et al.</i> , 2004)	$> 1 \text{ ng/g}$ to $< 0.1 \mu\text{g/g}$	$\geq 10 \text{ ng/g}$ (TEGA)	$\sim 100 \text{ ng/cm}^2$ or best effort	$< 100\text{--}400 \text{ ng/cm}^2$ (scoop sides, TEGA–EGA cover) (Anderson, 2006)	Unknown
MSL (Eigenbrode <i>et al.</i> , 2013a; Glavin <i>et al.</i> , 2013; Leshin <i>et al.</i> , 2013; Blakkolb <i>et al.</i> , 2014; Ming <i>et al.</i> , 2014)	$\leq 40 \text{ ng/g}$	Sub-ng/g (SAM)	$\leq 100 \text{ ng/cm}^2$ at launch (approximately $< 0.4 \text{ ng/cm}^2$ after <i>in situ</i> dilution cleaning)	$\leq 20\text{--}40 \text{ ng/cm}^2$ at launch, approximately 0.4 ng/cm ² after dilution cleaning at Mars ^a	$\leq 40 \text{ ng/g}$ TOC (Eigenbrode <i>et al.</i> , 2013a; Glavin <i>et al.</i> , 2014; Leshin <i>et al.</i> , 2013; Ming <i>et al.</i> , 2014)
OSIRIS-Rex	Better than 1 $\mu\text{g/g}$ TOC				—
ExoMars	<ul style="list-style-type: none"> Material from biological sources $\leq 50 \text{ ng/g}$ Monomers of Kapton, Mylar, and PTFE $\leq 500 \text{ ng/g}$ Fluorinated technical lubricants $\leq 500 \text{ ng/g}$ Any other organic compound $\leq 50 \text{ ng/g}$ 	10 ng of NVR/cm ² (MOMA instrument)	180 ng/cm ²	—	—

^aValue calculated and not measured.
EGA, evolved gas analysis; MAE, Molecular Analysis Experiment; PHX, Phoenix.

(2) everything else (deceased organisms and their remains, abiotic organics, etc.) that can be studied via the molecular composition of organic materials. The former class is narrow in the sense that the vast majority of terrestrial microbes cannot be cultured using existing techniques. Culturing requires prior knowledge of the necessary requirements for metabolism, and is inherently Earth-centric. Therefore, a culture-based approach to detecting martian life would be a thoroughly inadequate approach for assessing the presence of martian organisms in returned samples.

In contrast to cultivation, measurements of molecular composition are able to detect not just living organisms, but also dead organisms, degraded fossil relicts of ancient life (Briggs and Summons, 2014), and organic molecules arising from abiotic or “prebiotic” processes (Brasier *et al.*, 2002; Tice and Lowe, 2004; McCollom and Seewald, 2007; House *et al.*, 2013). The OCP concludes that investigating carbon-based organic compounds would be one of the more fruitful approaches for seeking potential signs of life in returned samples as opposed to culture-based approaches.

Finding #2: Detection and characterization at the molecular level of indigenous organic compounds are of fundamental and critical importance to the searches for ancient and extant life in martian samples.

2.3.2. Analytical method limits of detection and contamination limits. The OCP was asked to consider detection limits of current laboratory analytical methods as a basis for establishing allowable contamination levels. In reviewing the literature, and based on our collective experience, we find that some modern analytical methods can detect vanishingly small quantities of organic molecules. Moreover, the overall detection limit of an instrument or method is not always clear-cut, being dependent upon such factors as sample preparation, target molecule, and matrix effects. Nonetheless, some methods that target certain molecules can have detection limits of parts per trillion (ppt) or lower (see Appendix 4), and it is reasonable to expect limits to further decrease in the future. For comparison, a ppt detection limit is about 3 orders of magnitude lower than that of the MSL SAM instrument.

The OCP concluded that it would be extremely difficult—and most likely impossible within realistic limits of cost and risk—to deliver martian samples to Earth *and* to get them analyzed, in a way that organic contamination levels were below the levels of the most sensitive possible organic-measuring instrument. With such low detection limits, we consider it inevitable that some level of terrestrial contaminants would be detected by future sample measurements. However, the key question for OCP is not whether it would be technically possible to return samples in such a state of cleanliness, but whether samples in that state are necessary to achieve our sample-related objectives.

Investigating samples that have measurable amounts of contamination does not necessarily mean that the science objectives related to organic chemistry in returned samples are in jeopardy. There are well-understood approaches to recognizing and analyzing indigenous organic matter in the presence of detectable contamination. These may include the molecular structure, chirality, isotopic composition, geologic context, and spatial distribution of compounds (see sidebar).

Some approaches to distinguishing signal from contamination:

1. Studying spatial distribution in relation to geologic features (*e.g.*, McDonald and Bada, 1995; Allwood *et al.*, 2009, 2013, Steele *et al.*, 2012). This means it is important to maintain as much as possible the physical integrity of the samples, such that such spatial relationships can be recognized (this may also involve *in situ* observations by rover instruments).
2. Differentiating between organic molecules that are in inclusions inside minerals versus on the surface of minerals (*e.g.*, Steele *et al.*, 2012). Here the premise is that the surficial (and therefore presumed to be contaminant) organics can be driven off by raising the temperature of the sample, and that the organics in the inclusions can be uniquely analyzed separately. If the organics are inside minerals, the organics were likely indigenous to the sample before collection.
3. Comparing the molecular composition of organics to known contaminants from the sampling system (*e.g.*, Grosjean and Logan, 2007; Hallmann *et al.*, 2011; Steele *et al.*, 2012).
4. Measuring the isotopic composition of organics, which may distinguish Mars versus Earth in respect to their carbon, nitrogen, and hydrogen isotopic compositions (*e.g.*, Leshin *et al.*, 1996, 2013; Huang *et al.*, 2005; Steele *et al.*, 2013).
5. As suggested by Bayesian logic, focus analyses on those samples with the highest probability of containing indigenous organic carbon (Sephton and Carter, 2014).

Studies of meteorites and ancient terrestrial organics show that it is possible in many cases to recognize indigenous organics and interpret their origin, despite the presence of considerable terrestrial contamination (*e.g.*, Callahan *et al.*, 2013). On the other hand, there is always some risk that contamination above detection limits would prevent determination of the materials of interest, so lower is clearly better.

It was outside the scope of the OCP's deliberations to evaluate the engineering consequences (cost, risk, schedule, complexity) of delivering samples at progressively cleaner levels, and comparing those consequences to the scientific benefits of analyzing progressively cleaner samples. However, the OCP concludes that the most prudent way to proceed in the absence of such a study is to assume that it is not practically possible to prevent organic contamination that exceeds state-of-the-art instrument detection limits. *We should assume the presence of a certain minimum amount of detectable organic contamination, and plan ways to discriminate such contaminants from indigenous signals.*

Major Finding #3: Because of the sensitivity of modern analytical instruments, we must accept that we would not be able to reduce *all* organic contaminants to non-detectable levels in all analytical techniques. Fully characterizing this residual contamination is essential.

2.3.3. Not all contaminants are equal. One approach to limiting organic contamination of the returned samples is to adopt limits on TOC contamination. However, certain

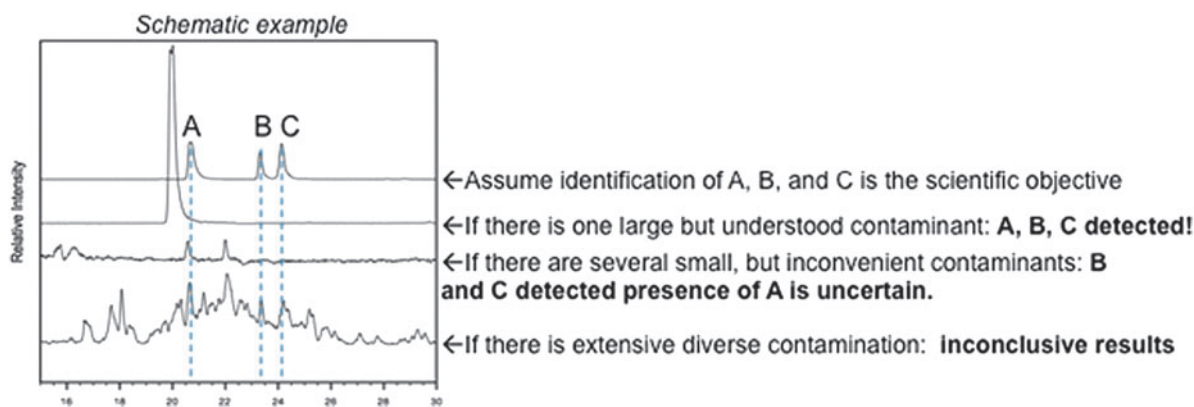


FIG. 4. Complex contaminant profiles are, in general, much worse than a few well-characterized contaminants.

contaminants can be more problematic than others if they directly interfere with analytes of interest. Without knowing the chemistry of the returned samples, it is difficult to propose specific molecules to avoid. Nonetheless, complex contaminant profiles generally make it much more difficult to detect target analytes than a few well-characterized contaminants. Therefore, both the complexity of contaminating compounds as well as the total amount of organic contamination (Fig. 4) can affect the interpretability of the data.

Major Finding #4: Reducing those specific contaminants that interfere with compounds of scientific interest is as important as reducing the total contamination burden.

2.3.4. Contamination control versus contamination knowledge. The Panel believes it is important to distinguish between the concepts of “contamination control” and “contamination knowledge.” The former represents the efforts needed to reduce contamination on the spacecraft (and eventually the samples), and to maintain that level of cleanliness (Fig. 5). It is inherently part of the project design, engineering, and fabrication effort, and is implemented to achieve quantitative requirements (but generally no more). In contrast, contamination knowledge represents our need to understand in as much detail as possible what remains on—or is added to—the spacecraft. It is performed by the project team, but would provide foundational knowledge for the

science team studying the samples. In many ways it should be considered as the first stage of scientific investigation. It has neither easily quantifiable limits, nor easily proscribed methodology. Indeed, when conducted to its fullest extent, it should more closely resemble scientific research than engineering design and testing.

Two important points emerge from this discussion. The first is that both contamination control (strategies #1 and #3 in Fig. 5) and contamination knowledge (strategies #2 and #5 in Fig. 5) are necessary and complementary. The second is that they would likely require fundamentally different approaches. Adequate consideration should be given to the necessary personnel, resources, and strategies required for both to be successful.

Finding #5: Our ability to correctly interpret data from partially contaminated samples correctly depends on a combination of (1) minimizing contamination at the start, (2) characterizing residual contamination, and (3) minimizing and characterizing recontamination. All three are equally important.

2.4. Science and PP objectives both drive need for organic analyses

Some of the scientific and PP objectives of MSR would both rely heavily on careful measurements of organic

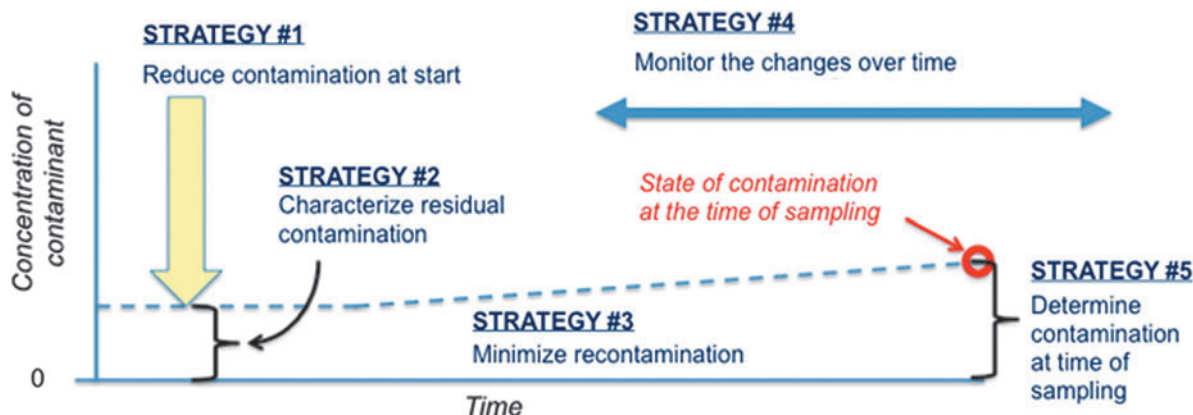


FIG. 5. Graphic representation of the elements of a viable organic contamination management plan for Mars 2020.

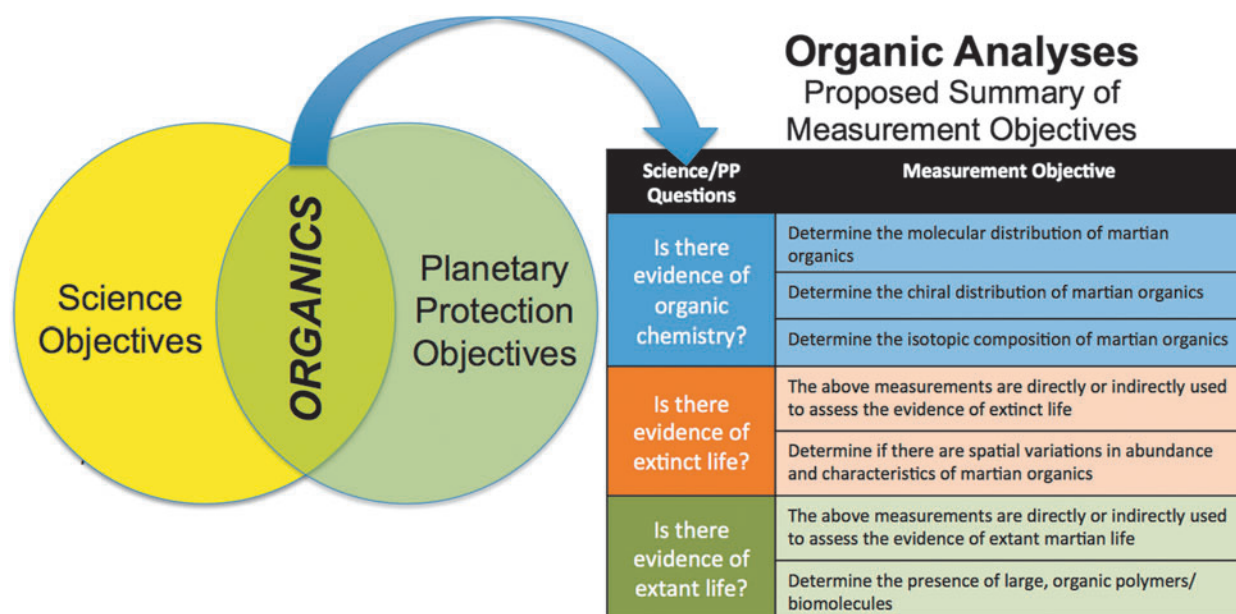


FIG. 6. Science and PP objectives would both drive the need for analysis of potential returned samples for organic molecules.

molecules in the returned samples (Fig. 6). There are different ways of organizing the way various interpreters think about the data [*e.g.*, the null hypothesis (see Kminek *et al.*, 2014)], but regardless of how the hypotheses are structured, all would make use of the same analytic data from the samples. Once samples are available for Earth-based analysis, they would be analyzed for their organic contents to the maximum degree possible. For both science and PP applications, a central part of the problem is to reliably interpret the geochemical/biological context of organic molecules on Mars. Whether or not they are indigenous to Mars is a key interpretation, and for which it would be very important to understand the degree of definitiveness. *Given that sample mass would be limited, we anticipate that it would neither be possible nor necessary to make separate measurements by “science” and “planetary protection” teams—both would have a shared need for accurate data from precious samples.*

In this context, the OCP carefully considered ways to organize a set of measurement goals related to organic molecules in any future returned samples that would constitute an approach that may be useful for both science investigators and PP technical evaluators (Fig. 6). A key uncertainty lies in the fact that the samples would arrive in terrestrial laboratories as inadequately characterized objects—we would have at best fragmentary information about the concentration and identity of contained organic molecules. This leads to two logical phases of investigation (illustrated by the upper and lower parts of the simple table in Fig. 6): (1) Do the samples contain organic compounds?, and (2) What is the nature and origin of any compounds discovered? The purpose of pointing out this intrinsic sequential approach is that the instruments and detection limits are somewhat different in the two phases, as are the sensitivities to organic contamination (all amplified in the next section). It is not our intent to comment on the important details that make up the life detection and hazard assessment protocols, both of which are well out-

side the scope of the Panel. However, we note that biomolecular organic analyses are now sufficiently sensitive to detect even the smallest possibly viable contaminant populations.

Major Finding #6: A key subset of the objectives of both science and PP could be met by a common set of analyses of organic molecules in returned samples. Although interpreters for science and PP would use the data in somewhat different ways, their need for accurate, high-precision data would be identical.

3. Sample-Based Investigations and Measurements

As requested by its charter, the OCP considered in detail the types of analytical measurements and their dependent instrumentation that might be employed in the investigation of returned samples. As discussed above, OCP’s purpose was to think through the logical ways of generating organic molecular inputs to PP’s test protocol (see Rummel *et al.*, 2002, and subsequent revisions), and to the scientific queries related to astrobiology. There are a very large number of potential instruments and methods that might be used to study samples returned from Mars. Furthermore, by the time samples are available for analysis on Earth, we can expect to see significant developments in both instrument capability (including new methodologies) and biochemical and geochemical knowledge. Current (2014) technology has the means to analyze millions of organic compounds via hundreds of different methods. In the future, when the samples are to be returned, presumably more analytical capabilities would exist. Limited sample mass would constrain the viability of various methods, as would the potential need for continued sample containment depending upon PP findings. It is therefore impossible for this Panel to agree on all specific target analytes or methods that would practically

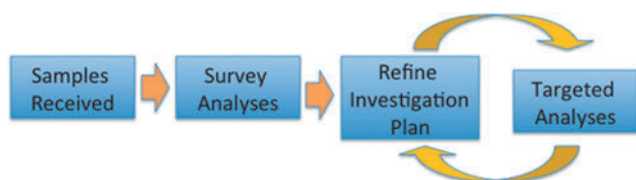


FIG. 7. Diagrammatic illustration of the relationship between early survey measurements and later targeted analyses.

and viably bound contamination needs. Moreover, we feel it is unwise for us to (even attempt to) predict which methodologies would be most appropriate or useful many years in the future, before anything is known about the putative samples.

Major Finding #7: A huge diversity of techniques for organic analysis exists as of 2014. More will be invented. Not all potential measurements would be possible on returned Mars samples given limited sample mass and potential containment requirements, nor would all be needed.

3.1. Need for early survey measurements

Because of the wide spectrum of available instrumentation, and the unknown character of the samples, it would not be possible to describe in advance a specific analytical plan for each sample. The investigation pathways would, of necessity, need to be driven by discovery (Fig. 7). The analytical scheme for any given sample would be contingent on its size, organic content, environmental and stratigraphic context, lithology, surface exposure age, thermal history, etc. Different samples may take quite different analytical pathways. It is for these reasons, as well as those of PP, that initial survey measurements would be needed to *determine whether organics are present and to provide first-pass characterization*. This information would then be used to prioritize (presumably via peer-reviewed proposals) a more targeted series of measurements geared toward more specific research objectives. Given the recommended integration of science and PP measurements, early survey measurements would need to be performed while the samples are still isolated in the SRF, which would pose technological challenges. Ensuring that the SRF would be capable of supporting such survey measurements should thus be a key consideration in its design.

Finding #8: The course of organic investigation for returned martian samples would, of necessity, be one of discovery and iterative refinement. Effective early survey measurements on returned samples are necessary to establish the full investigation plan for each sample.

3.2. Potential analytical methods for returned samples

As part of its research, the OCP compiled a list of analytical methods that are typically employed to investigate samples for organic constituents (Table 4). A confusing but important aspect to consider in comparing these methods concerns the units of measurement. For example, different

techniques would yield results that are properly expressed in units of mass/area, mass/mass, mass/volume, ppb, etc. Depending on details of sample introduction, some of the instruments and techniques are inherently sensitive to the total mass of molecules in a sample aliquot (e.g., mass spectrometry), while others are sensitive to their concentration per unit area or volume (e.g., optical spectroscopy). As a concrete example, the absolute detection limits for mass spectrometers are most properly expressed in units of mass (nanogram) or moles (nanomole). Thus, to apply a mass spectrometric detection limit to a returned sample, we require knowledge of how much sample would be assayed to derive ppm ($\mu\text{g/g}$) or ppb (ng/g) by weight. For surfaces, the area of sample to be assayed is needed to derive a contamination limit (e.g., ng/cm^2).

A further complication is that when we specify the mass of an amino acid (e.g., 1 ng of glycine) detected in a mass spectrometry assay, we are specifying the total mass of that compound. Knowledge of the molecular formula then allows conversion to an equivalent mass of carbon. For example, a molecule of squalene (common contaminant from fingerprints) weighs almost six times as much as a molecule of glycine and, molecule for molecule, has 15 times more carbon. Considerations such as these must be factored into understanding the relationships between measurements such as TOC (which measures carbon mass) and those such as analyses of specific amino acids (which target total analyte mass).

One of the key outcomes of this exercise was recognition that there are *so many* potential measurement techniques, in many cases with extraordinarily low detection limits, that it is impossible to avoid contamination that would affect *any* of them. We cannot know in advance every contaminant that *might* matter, but we can predict ones that *definitely would* matter (see discussion in Section 4). Without some means to narrow our focus on a smaller subset of methods, they cannot serve as a useful basis for establishing appropriate limits on contamination. Any attempt to simultaneously consider all such methods must necessarily reach the conclusion that virtually all organic compounds must be limited to vanishingly small concentrations.

As a means to broach this apparent conundrum, the OCP decided to parse prospective analytical methods into those most suitable for use in initial survey measurements (hereafter, referred to as “survey methods”) versus those more suitable for targeted measurements (in the sense of Fig. 7; “targeted methods”). This is an admittedly arbitrary distinction, in that many instruments could be used to make either survey or targeted analyses. For example, the same mass spectrometer operating in full-scan mode can provide a useful survey of what is present, whereas when operating in selected-ion or tandem mass spectrometry (MS-MS) mode would provide a more sensitive and detailed picture of particular analytes. Nevertheless, this distinction is useful in that we know we would utilize survey methods on the returned samples, but the use of targeted methods would be contingent on what is present. Ideal characteristics of survey methods would be (1) broad sensitivity to a wide range of organic analytes, (2) minimal sample consumption, and (3) suitability for use in a containment facility. The latter consideration rules out, for example, synchrotron x-ray spectroscopy.

TABLE 4. SCIENCE/PLANETARY PROTECTION QUESTIONS, MEASUREMENT OBJECTIVES, AND INSTRUMENT TECHNIQUES

<i>Science/PP questions</i>	<i>Measurement objective</i>	<i>Required measurement</i>	<i>Technique/instrument^d</i>
Is there evidence of organic chemistry?	Determine the molecular distribution of martian organics	Assess the functional groups present	Infrared spectroscopy (reflectance, transmittance, glowbar or synchrotron FTIR)
		Assess the aromatic compounds present	Deep UV Raman/fluorescence spectroscopy
		Assess the volatile/semi-volatile species	X-ray absorption near edge structure (XANES)
		Assess the non-volatile species	Laser desorption-mass spectrometry (LD-MS, L2MS, μ L2MS)
	Determine the chiral distribution of martian organics	Assess the enantiomeric ratios of amino acids, amines, carbonyls, etc.	Gas chromatography-mass spectrometry (GC-MS, GC/GC-MS, GC-TOF-MS, GC-QQQ-MS, etc.)
		Determine the stable isotopic ratio of molecules (C, H, N, O, and S)	Evolved gas analysis (EGA)
		See above	Bulk atmospheric pressure mass spectrometry (DART, DESI, infusion, etc.)
		Identification and spectral properties of submicron aggregates of organic matter	Liquid chromatography or supercritical fluid-mass spectrometry (LC-MS, SCF-MS, LC-TOF-MS, LC-FT-MS, etc.)
	The above measurements are directly or indirectly used to assess the evidence of extant life.	See above	Time-of-flight secondary ion mass spectrometry (ToF-SIMS)
		Determine the presence of large, organic polymers/biomolecules	Solid-state nuclear magnetic resonance spectrometry (NMR)
Is there evidence of extant life?	The above measurements are directly or indirectly used to assess the evidence of extant life.	Assess the enantiomeric ratios of amino acids, amines, carbonyls, etc.	LC-MS or GC-MS optimized for chiral separation
		Determine the stable isotopic ratio of molecules (C, H, N, O, and S)	Ion microprobe [<i>e.g.</i> , nano-secondary ion mass spectrometry (nano-SIMS)]
		See above	Tunable laser spectroscopy (TLS)
		Identification and spectral properties of submicron aggregates of organic matter	Elemental analysis-isotopic ratio mass spectrometry (EA-IRMS, TC/EA-IRMS)
	The above measurements are directly or indirectly used to assess the evidence of extant life.	See above	Compound-specific isotope analysis (GC-IRMS, GC-ICPMS)
		See above	Most of the above techniques are necessary contributors to this question.
		See above	Confocal Raman spectroscopy at up to 360 nm micron spatial resolution
		See above	Deep UV Raman/fluorescence spectroscopy
	The above measurements are directly or indirectly used to assess the evidence of extant life.	See above	Confocal Raman spectroscopy at up to 360 nm micron spatial resolution
		See above	Scanning electron microscope/energy-dispersive X-ray spectroscopy (SEM/EDX)
Is there evidence of extant life?	The above measurements are directly or indirectly used to assess the evidence of extant life.	See above	Most of the above techniques are necessary contributors to this question.
		See above	Polymerase chain reaction (PCR)
		See above	Multiple displacement amplification (MDA)
		See above	Fluorescence imaging of fluorescently tagged compounds (FISH)
	The above measurements are directly or indirectly used to assess the evidence of extant life.	See above	Enzyme-linked immunosorbent assay (ELISA)
		See above	Limulus amoebocyte lysate (LAL) assay
		See above	Adenosine triphosphate (ATP) luminometry
		See above	Microbial plating assay
	The above measurements are directly or indirectly used to assess the evidence of extant life.	See above	Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)
		See above	Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)

^aSee Appendix 4 for instrument sensitivities, preparation requirements, and notes.

3.3. Survey versus targeted analytical methods

Most analytical methods exhibit an inherent trade-off between specificity and sensitivity. The ability to confidently detect and identify a given analyte is governed by the measured signal-to-noise (S/N) ratio. Signal is largely a function of the sample concentration and physics (ionization cross-section, quantum fluorescence yield, etc.), so the most effective way to boost sensitivity is to reduce noise. Noise in the context of a complicated, geologic sample is mostly determined by the number and abundance of *other* (*i.e.*, non-analyte) molecules being detected. The primary means to reduce noise (and so increase S/N) is thus to screen out signals from everything other than the analyte of interest (*e.g.*, only looking at one particular mass or wavelength). In other words, the act of simultaneously looking for everything would generally decrease our ability (sensitivity) to detect any one thing in particular. We conclude that survey analytical methods are likely to be inherently less sensitive than are targeted analytical methods.

Two categories of likely survey methods can be recognized. Surface imaging and surface spectroscopic/spectrometric techniques are minimally destructive and so likely to be used at an early stage. Their strengths include minimal sample consumption, *in situ* analysis with excellent spatial resolution, and broad sensitivity to many analytes. *In situ* observations also generate context for the organics that can be related to the bulk measurements. However, identifying and quantifying specific individual molecules present in complex mixtures are limited using these techniques, and so other methods are needed for complete molecular characterization. Mass spectrometric techniques used with bulk or extracted samples are destructive of samples, but currently provide the best ability to look sensitively for a broad range of organics, and to identify them and their specific characteristics (*e.g.*, isomeric form). Most recent studies of meteorites have used mass spectrometry in some form.

Ultimately, some combination of non-destructive and destructive techniques would likely be used to establish the survey analyses of samples.

Based on the information compiled in Appendix 4, the OCP concludes that the detection limits for surface spectroscopy are typically in the ppm range, whereas for mass spectrometry, detection limits are in the ppb range. An important implication is that surface spectroscopic measurements should not drive the contamination limits. In contrast, mass spectrometric methods cover a very wide range of potential analytes (and indeed are valuable for this ability), and their detection limits are sufficient to identify the principal organic molecular components in geological samples at the nanogram level.

“Targeted” methods for organic characterization would be those that look more sensitively for a reduced number of specific components. The decision of whether or not to employ any particular targeted method would be based on a combination of what is known to be present in the samples, perceived importance of the target analytes, sample availability, and other factors. Some targeted analyses (*e.g.*, for amino acids) might be deemed so essential that they would be undertaken no matter what is found by initial surveys. Others (*e.g.*, for hydrocarbons) may not. Regardless, knowledge from the survey measurements of which compound types are present would enable prioritization of which targeted methods to use, and so are more essential than targeted methods that may or may not be used. The OCP therefore decided to focus on survey methods for the purpose of establishing tractable contamination limits (Figs. 7 and 8).

3.4. Survey analytical methods

The most likely initial survey measurements would employ one or more of a range of surface spectroscopy tools. In

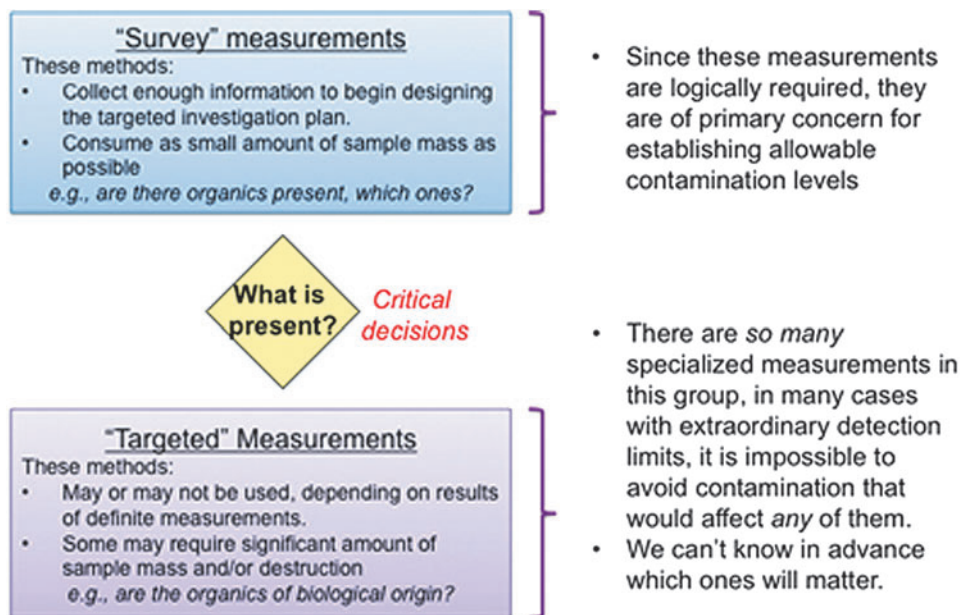


FIG. 8. A two-step analytic process for organics—survey versus targeted.

part, this would be driven by the need for the initial investigations of returned samples to be non-destructive. These include FTIR, IR reflectance spectroscopy, confocal Raman spectroscopy, and deep UV Raman/fluorescence spectroscopy. These methods are non-destructive, but in most cases do not allow the identification of individual molecules in complex mixtures. On the other hand, they generally do not require significant sample preparation and work at small spatial scales. Therefore, they can be very useful for detecting organic carbon aggregates on the surface or within inclusions in samples.

Surface spectroscopic techniques can be complementary and can precisely survey a sample for surface organic contamination, including contaminant heterogeneity and general type. In decreasing order of organic sensitivity, these are (1) deep UV Raman and fluorescence spectroscopy, (2) confocal Raman spectroscopy, (3) FTIR spectroscopy, and (4) IR reflectance spectroscopy. In conjunction with their capabilities for organics detection, each has strengths for mineralogical assessment, which is critical not only for distinguishing between native organics and contamination, but also for determining detection limits and detection depths.

Finding #9: A small number of currently known measurement methods is sufficient to provide the survey information required. These measurements would then become essential input into deciding which targeted methods should be applied.

4. Sample-Based Contaminants of Concern

One of the primary goals of the OCP was to propose quantitative limits for organic contamination that would be considered acceptable in the returned geologic samples. The primary purpose of such limits is to ensure that excessive organic contamination does not thwart either the scientific goals for sample return or the PP requirements needed to release the samples to the scientific community. Translating these goals into specific, quantitative limits is of course complicated, so the Panel discussed and researched a number of related concepts and issues, including the proposals and rationale of previous work on this subject. These considerations lead us to propose limits for individual organic compounds (Section 4.2) and for TOC (Section 4.3) and to discuss the impact of particulate organic matter (Section 4.4).

There was a general consensus among the panelists that we do not currently know enough about martian organic geochemistry to reach a single “right” answer to this charge, either with respect to *which* contaminants or *how much* should be allowed. Indeed, the entire purpose of returning martian rocks is to discover what might be there, and so the tolerable types and levels of contamination are in some sense unknowable at this time. This fact was also emphasized by the earlier SDT report (Mustard *et al.*, 2013). While we believe the particular strategy we have used to propose limits is scientifically sound, it is not the only possible outcome of such an exercise. Other groups of experts, weighting various considerations differently, might reasonably reach a different set of conclusions that are no less (or more) defensible than the ones offered here.

Finding #10: Because we fundamentally do not know what organics would be present on Mars, it is currently impossible to precisely determine what levels of contamination would be necessary in returned samples. There is thus significant uncertainty (in both directions) associated with the proposed limits.

4.1. General considerations

4.1.1. Selection criteria for choosing contaminants of concern. From a purely analytical standpoint, the best approach to setting contamination limits requires prior knowledge of the specific analytes and their concentrations that we hope or expect to measure. Based on consideration of appropriate S/N thresholds, quantitative limits on background contamination could then be derived for each analyte of interest (*e.g.*, Peters *et al.*, 1974). In our present case, however, we know neither the compounds we expect to find, nor the lower limits of their concentrations. This knowledge gap led us to consider other criteria for setting limits. A second possibility, mentioned by the OCP charter, would be to set contamination limits below the detection limits for analytical methods expected to be used. This is a very conservative approach, but as described in Section 3 there are too many analytical methods that could potentially be employed for us to plausibly consider them all, and some especially targeted techniques have extremely good (down to single molecule) sensitivity.

A third criterion that was discussed is the level of cleanliness that can reasonably be achieved in constructing sampling hardware using existing technology and methodology. There was considerable disagreement on the Panel about how (or whether) to employ such criteria. On one hand, the extraordinary effort required to return samples from Mars constitutes a powerful argument that limits should be driven solely by scientific goals, regardless of whether or not they are currently achievable. If not, the argument goes, then we should develop new technology that can achieve the required limits before launching the mission. On the other hand, our deep uncertainty about

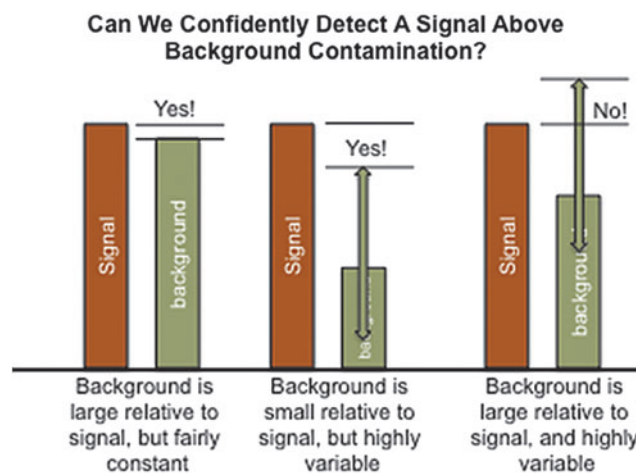


FIG. 9. A diagram showing how different levels of signal and background support or deny a successful measurement. The vertical arrows indicate variability in background levels.

quantitatively what to expect in returned samples makes a specific definition of limits for what is scientifically *required* virtually impossible. The Panel has tried to keep in mind the fact that although it may be technically possible to return samples cleaner than a certain level, this does not constitute an argument that such cleanliness is necessary.

4.1.2. S/N threshold for acceptable contamination. A common misconception is that robust analytical measurements require background (blank) levels of a particular analyte to be well below those one is trying to measure. As an example, measuring glycine with an S/N level of 3 (a commonly accepted threshold) might be misconstrued as meaning glycine concentration in the blank must be three-fold lower than that in the sample. This is incorrect. Our ability to confidently resolve the presence or absence of an analyte requires only that we can determine at some statistical confidence interval (say, 99.7% or 3σ) that the measured concentration is greater than that of the blank. Thus, as illustrated in Fig. 9, we need to know both the concentration of a particular contaminant in our blank and the uncertainty in our knowledge of that concentration. This uncertainty could result from incomplete characterization, or from physical variability (whether spatial or temporal) in its abundance, or both. This leads to two important points. First, contaminant concentrations do not necessarily need to be lower than detection limits for a measurement to be successful. Second, a stable and well-characterized background is as important as a low background.

Finding #11: Confirming a measurement to be statistically significant does not necessarily require that contamination be below the detection limit, only that it be acceptably low, stable, and well characterized. Consequently, the contamination knowledge program should address the composition, abundance, and variability of organic molecules present on the spacecraft.

In practical terms, the *variability* of background contamination is very difficult to control, and is likely to be strongly dependent on the particular analyte. Certain volatile organic compounds that are ubiquitous in clean room air as a result of degassing from permanent equipment (flooring, duct work, paint, etc.) might prove to be present on sampling hardware at very predictable levels. Other compounds, for example, those present in cleaning products used intermittently, might be much more variable. Particulate organic contamination would have a statistical spatial variability determined by its Poisson mean concentration. Obviously, the more variable a particular contaminant is, the lower its average concentration must be to ensure that a particular sample concentration could be confidently measured.

Variability is also influenced by the scale of sampling. For example, contaminant concentrations can vary between samples, as well as spatially within a single sample. An organic particle represents a relatively large *local* concentration, but if there are few such particles, then could also represent a small concentration averaged over the entire sample. In recognition of such issues, we recommend that the appropriate scale for averaging contamination levels to

compare with specified limits is that of the individual sample core. In other words, if the specified limit is 1 ng/g, then each and every sample that is collected should meet that limit (rather than the average of all samples). Conversely, we do not recommend that subportions or aliquots of any given sample be required to fall below the limit. As long as the average concentration for a sample falls below the limit, then portions of that sample could reasonably exceed the limit.

4.1.3. Limits for TOC, individual molecules, or particles. The Panel considered at length the question of whether specific contamination limits are required for every compound of interest, or if a single limit on TOC would be sufficient. The Panel is also aware of the daunting challenge to actually achieve even ballpark estimates of TOC abundances. Given the potential co-existence of CO₂-yielding minerals together with the apparent near-ubiquity of perchlorate, which can react with organics to yield CO₂, it is far from certain how an accurate TOC measurement might be achieved in practice. There was agreement that measurements of certain individual compounds, for example, amino acids, are of such importance to the mission that they should be explicitly protected (*i.e.*, we should be certain the level of contamination of *those specific compounds* is below some threshold). The question then becomes whether to do so through limits for each analyte, or through a single limit on TOC set at (or below) the lowest level desired for any single compound. The former is potentially burdensome from an analytical perspective, given the number of possible analytes of interest, but it does allow limits on TOC to be relaxed somewhat. The latter is analytically simpler and more conservative, but the lower TOC levels required are potentially harder (and costlier) to achieve.

As a concrete example of this trade-off, if proposed contaminant limits for all analytes of interest range between 1 and 10 ng/g per compound (see Section 4.2 below), setting a TOC limit of 1 ng/g would guarantee that all individual limits had been met without having to measure each one. This is a very conservative approach, because most individual compounds would then be far below the required limits, and TOC contamination would likely be orders of magnitude below what is required to measure TOC. But, it has the benefit of simplicity. The OCP was not charged with determining the level of effort or costs associated with an attempt to implement such a limit, and this is an option the Mars 2020 Project may choose to consider.

Based on these considerations, the panel decided to propose limits for individual compounds of concern, while also proposing a TOC limit that would serve as a blanket insurance policy. This hybrid approach was previously recognized by the OCSSP (Mahaffy *et al.*, 2004), and was proposed by both them and the subsequent MSR-SSG II panels. This is a somewhat less conservative approach than that of a single TOC limit, but should provide equivalent protection of scientific measurements while being potentially easier to achieve. The primary perceived drawbacks are the increased number of analyses required to ensure compliance with limits for many different compounds and, depending on the quantitative levels, may allow a greater amount of total organic contamination to be present. These

more specific analyses also probably can overcome the severe challenges indicated above for TOC analyses.

4.1.4. Alive versus dead microbial contamination. Given that sample return missions would be especially concerned with looking for both extant and extinct martian life, and that we do not know how similar Mars-based biochemistry could be to that of terrestrial organisms, there was a general consensus that the strategy for detecting life on returned samples should not rely solely on current bioanalytic techniques [*i.e.*, those based on deoxyribonucleic acid (DNA) amplification, antibody/antigen recognition, ATP, etc., that are particular to life as we know it]. That is not to say that such analyses will not be used or useful, simply that they are not solely sufficient to detect martian organisms. Our thinking has thus been dominated by chemical measurements that identify and quantify the basic organic molecules that could be components of life. In general, these measurements cannot distinguish between molecules that are part of a living cell versus those that are not. From the standpoint of measurements of individual organic chemicals, we do not draw any distinctions between contaminants derived from living versus dead sources, as both have equivalent impacts at the molecular level.

Even though it would be very valuable to be able to distinguish live from dead Earth-sourced microbial contamination, particularly for PP needs, the OCP is not aware of molecular analytic methods for drawing this distinction. An issue with trying to distinguish live from dead using culture-based growth experiments is that a large fraction of the live microbes (commonly estimated at 99%) cannot be grown in the laboratory, and their response is therefore the same as for that of dead organisms. Note that since live and dead Earth-sourced microbial contaminants could both contribute organic molecules to a returned sample, sample sterilization (*i.e.*, converting live ones to dead ones) would not change the overall state of molecular contamination, so this topic was not discussed by OCP.

Finding #12: It is not currently possible to reliably and easily distinguish organics in living versus nonliving matter using molecular methods; thus both can be treated as equivalent from the standpoint of chemical contamination.

4.1.5. The possibility of reproduction of Earth-sourced microbial contaminants in sealed sample tubes. The question was raised during OCP's discussions about whether live terrestrial microbial contaminants could reproduce inside a sealed sample tube, thereby significantly altering both the forms of a sample's organic constituents and the state of "Earth-sourced" contamination. The probability of this outcome is the joint probability that one or more live organisms ends up in a particular sample, the probability that the minimum conditions for biological activity are exceeded, and the probability that the organism becomes active. OCP finds that it could not credibly penetrate this topic within the time constraints of this study. The conditions for cell division have recently been studied by Rummel *et al.* (2014) and have been found to be primarily dependent (at least for Mars ap-

plications) on temperature and water activity. Active refrigeration of the samples on Mars is unrealistic, and the water activity would reflect the nature of the samples (which is currently completely undefined). Thus, the only variable related to this that can be effectively managed is the probability of live terrestrial microbes in the samples. As a good experimental practice, therefore, OCP recommends that Mars 2020 be designed so that the sample tubes would be sterilized, and so that they could be sealed with a sample inside with a probability of less than $1\text{E-}2$ of a single live terrestrial organism per sample. (The quantitative figure of $1\text{E-}2$ was proposed by the then Planetary Protection Officer in a 1999 letter to the project manager of the 2003/2005 MSR Project, and this has triggered quite a bit of debate since. This figure appears sufficient to meet science needs, but OCP is not in a position to comment on PP needs in this area.)

4.2. Considerations related to specific contaminants

The OCP considered two distinct but related questions in relation to contamination by individual organic molecules: which ones do we care about, and at what level? Although the two are intimately linked, and need to be (and have been) considered iteratively, we separate them here for the purpose of clarity.

4.2.1. Which contaminants?. There are a vast number of organic molecules that exist on Earth, and might exist on Mars. A substantial subset of these molecules exists in carbonaceous meteorites. Our level of interest in these molecules from the point of view of Mars exploration spans the range from those involved in all life and in prebiotic synthesis (*e.g.*, amino acids) to those that are clearly man-made (*e.g.*, PTFE, aka Teflon). Unfortunately, most organic molecules fall into the very substantial gray area between these extremes (*i.e.*, we can be neither confident nor dismissive of their association with martian life). Prioritizing the relative scientific importance of molecules is highly subjective, as demonstrated by the divergent rankings produced by the OCSSG and MSR-SSG II reports. Even those compounds that are clearly man-made have the potential for interfering with sensitive analyses of more interesting molecules, for example, due to spectral interference, chromatographic co-elution, isobaric interference, etc. There was thus a strong consensus by the Panel that we cannot afford to ignore any type of organic compounds—at some level, we must care about them all, including man-made molecules.

This leads to a logical problem, in that the vast number of organic compounds makes it virtually impossible to measure them all, in order to verify an acceptably low concentration in the contaminant load. One possible approach would be to set very conservative TOC limits, as discussed above (Section 4.1.3), which would constrain the sum of all organic contaminants—any single contaminant getting wildly out of control could be detected this way. Note that the setting of a TOC constraint does not relieve the burden of characterizing the residual compounds. A second approach, which we believe has several benefits, is to create two tiers of individual contaminants. The first (Tier I) constitute those molecules that are likely to be most important to the science goals of the mission (*i.e.*, those that could be indicative of

martian and/or terrestrial life). Our recommendation is that levels of contamination by these molecules should be actively monitored and controlled during spacecraft assembly. The second group (Tier II) then comprises all other known organic molecules (see Section 2.1.4 for our definition of what constitutes “organic”), and can presumably be limited to some higher level of contamination than Tier I, and monitored with less rigor. The great difficulty with this approach, of course, lies in deciding which molecules belong in Tier I versus II. The OCP addressed this difficulty both through discussions among the Panel and by soliciting input from outside reviewers. Several compounds were added to respond to external critiques.

Our general criteria for assembling the list of Tier-I molecules is as follows: (1) molecules known to be important to terrestrial life, (2) molecular fossils of terrestrial life (*e.g.*, sedimentary hydrocarbons derived from ancient biomolecules, such as those that constitute ancient “biomarkers” on Earth), (3) molecules known to be present in carbonaceous meteorites and/or important to prebiotic chemistry, and (4) molecules that have already been tentatively detected in martian materials and are thus likely to be measured in returned samples [*e.g.*, chlorobenzene, polycyclic aromatic hydrocarbons (PAHs)]. It can be (and has been) argued that some molecules not used by terrestrial life might be central to martian biochemistry, and so belong in Tier I. However, we have no quantitative basis for choosing such potential exotic chemistries, and thus we have chosen not to speculate; this is a very slippery slope that would immediately lead to a huge increase in the number of targeted compounds, to the extent that the viability of our proposed two-tiered strategy is jeopardized. We also point out that Tier I does *not* include many compounds that are likely contaminants, such as solvents, cleaning agents, lubricants, plasticizers, etc., and it is assumed that such contaminants are easily recognizable as such. In essence, the Tier-I list comprises those compounds that we hope to find in our samples, not those that we hope not to find. This list is similar, although not identical, to that provided by the earlier OCSSG report (Table 2 and Section 2.4 in Mahaffy *et al.*, 2004).

Even with these criteria applied rather stringently, the list of prospective Tier-I analytes would still likely stretch into the thousands, which is large enough to make specific testing of all compounds prohibitive. This fact has been wrestled with by all of the previous groups working on the subject. The OCP thus proposed adopting the further simplification that a subset of these compounds would be representative of the entire group. This is equivalent to the assumption that Tier-I contaminants are likely to arrive on spacecraft surfaces via a few well-characterized vectors, namely, terrestrial life and/or petroleum products. Although the biomolecules that comprise terrestrial life do vary in their relative proportions, they do so within relatively narrow and well-understood ranges: if the amino acid alanine is present as a contaminant, it is likely to be accompanied (within an order of magnitude abundance) by valine, leucine, tyrosine, etc. Similarly, palmitic acid is the most common fatty acid, and thus is likely to be correlated with oleic acid, stearic acid, etc. Similarly, squalene is one of the most common neutral lipids. Other biochemicals (for example, sugars and non-polymeric nucleotides) have been left

off of Tier I following the same logic (*i.e.*, that their abundance should be roughly correlated with those of amino acids and fatty acids). We have included both amino acids and lipids in Tier I, despite the likelihood of their being correlated in most contamination vectors, due to their very different adsorption properties. The case of petroleum is more difficult, because the molecular (biomarker) composition of petroleum products varies more widely (Peters *et al.*, 2005). Nevertheless, we have selected just two common petroleum constituents for our Tier-I list. *n*-Heptadecane can be found in both leaf waxes and petroleum, and so covers two potential sources of contamination. Pristane, because of its unique structure, is presumably unique to terrestrial products and would be important in distinguishing, for example, hydrocarbons from terrestrial versus meteoritic sources (Peters *et al.*, 2005; Illing *et al.*, 2014). In the case of chlorinated organics liable to be present on Mars, we have simply confined our list to the two compound classes that have been reported to date [chloromethanes and chlorobenzene (Freissinet *et al.*, 2014; Ming *et al.*, 2014)]. By aggressively employing this strategy of representation and simplification, the Panel arrived at a list of 16 compounds to be monitored as Tier-I contaminants (Table 5).

The Panel acknowledges that this strategy is not risk-free. For example, by explicitly monitoring only alanine, glycine, and palmitic acid, we would not unambiguously guarantee that tyrosine, oleic acid, and glucose are present at equivalently low levels. Ensuring low levels of pristane would not by itself guarantee low levels of cholestane or hopane. Nevertheless, this compromise was seen as essential for arriving at a manageable list for explicit monitoring. The only reasonable alternative, in our view, is to limit all organic contamination (*i.e.*, TOC) to equivalently low levels, which has problems of its own (Section 4.1.3 above). We emphasize that our implicit goal in drawing up Table 5 is that all related compounds (*e.g.*, all proteinogenic amino acids, common lipids, nucleotides, sugars, hydrocarbon biomarkers, etc.) should be at similar or lower levels. If anomalous concentrations of such compounds are discovered by the project's contamination characterization program, they should be mitigated accordingly.

4.2.1.1. Tier-I contaminants. It is proposed that not more than 1 ng/g of any of these molecules sourced from Earth be allowed in or on the geological samples before they are analyzed. Most of the measurements listed in Table 5 have picogram detection limits.

4.2.2. Allowable levels of contamination. In order to arrive at plausible and defensible limits on individual organic contaminants, the panel considered three related criteria as described above and in Fig. 10. Of these, expected concentrations of targeted analytes are the most relevant to scientific goals, but are unfortunately also the least constrained. We therefore considered available data for all three criteria in the following sections. In doing so, we have made very broad generalizations about concentrations and detection limits for all organic compounds in all sample matrices. We point out that this is a very substantial oversimplification, for example, detection limits can vary by at least an order of magnitude between compounds or matrices

TABLE 5. PROPOSED LIST OF TIER-I CONTAMINANTS

<i>Contaminant class</i>	<i>Examples</i>	<i>Potential measurement methodology</i>	<i>Comments/justification</i>	<i>References</i>
Nucleic acid	DNA	Intercalation dye and hanging drop fluorimeter LC-MS	DNA is the universal signature for terrestrial life and, therefore, terrestrial contamination	Liu <i>et al.</i> (2013)
Spores	Dipicolinic acid	Fluorescence	Bacterial spores are the most recalcitrant form of terrestrial biota	Krásny <i>et al.</i> (2013)
Bacterial and fungal cell walls	<i>N</i> -Acetylglucosamine	LC-MS	Bacterial and fungal cell wall components may be detectable after the cell is destroyed.	Schleifer and Kandler (1972) Bartnicki-Garcia (1968)
Amino acids	Glycine	LC-MS	Glycine is the most abundant amino acid in nature; abundant in fingerprints.	Salazar <i>et al.</i> (2012)
Lipids	Alanine	LC-MS	Alanine is chiral and abundant.	
	Palmitic acid	GC-MS	Most common fatty acid in bacteria and eukarya	
	Squalene	GC-MS	Lipid common to all life; abundant in fingerprints	
Hydrocarbon biomarkers	Pristane	GC-MS	Common component of petroleum and, therefore, petroleum-derived aerosols	
Martian organics	Chlorobenzene	GC-MS	Need at least one likely Mars-derived organic compound. Chlorobenzene is a reaction product of aromatic carboxylic acids (<i>e.g.</i> , benzoic, phthalic) with perchlorate.	Benner <i>et al.</i> (2000) Biemann <i>et al.</i> (1977) Navarro-González <i>et al.</i> (2010)
	Dichloromethane	GC-MS	Identified by both Viking and MSL. May be terrestrial and/or martian	Biemann <i>et al.</i> (1977) Navarro-González <i>et al.</i> (2010)
PAHs	Naphthalene	GC-MS	Most abundant and readily detectable PAH. PAHs have been detected in ALH 84001 and DaG 476 and appear to be part of the aromatic inventory of martian igneous and possible biogenic processes. Should be monitored to avoid false-positive measurements	Clemett, <i>et al.</i> (1998) Steele <i>et al.</i> (2012)
Nitrogenous compound	Urea	LC-MS	Important to prebiotic chemistry	Esther <i>et al.</i> (2008) Hu <i>et al.</i> (1994)
Short-chain carboxylic acid	Acetic acid	GC-MS	Simple organic acid relevant to both biological and industrial contamination sources	
Polyhydroxy compound	Glycerol	GC-MS	Simple polyol relevant to both biological and industrial contamination sources	
Hydroxy carboxylic acid	Pyruvic acid	LC-MS or GC-MS	Metabolite of sugars and important metabolic intermediate	
Linear hydrocarbons	<i>n</i> -Heptacosane	GC-MS	Common industrial hydrocarbon contaminant	

(Watson and Sparkman, 2007). On the other hand, the Panel did not feel it was productive to undertake the huge effort required to develop detection limits and cleaning standards for every different class of analyte and sample material, given the large uncertainties in what we expect to find in the

final samples. The reader should therefore treat the following discussion as best presumed, rather than precise values.

4.2.2.1. *What analyte concentrations do we expect?* Preliminary estimates of Mars *in situ* organic



FIG. 10. Determining allowable levels of contamination (see Sections 4.2.2.1–4.2.2.3).

concentrations and molecular distributions can be derived from four sources: studies of martian meteorites found on Earth, lander- and rover-based *in situ* measurements, analogous terrestrial environments, and estimates of delivery rates of meteoritic organics. Studies of martian meteorites are the most detailed and comprehensive of *in situ* martian concentrations, but suffer from at least one significant drawback; existing meteoritic samples exclusively represent igneous martian rocks, which are not the principal rock types likely to be examined for life detection on Mars (Grotzinger *et al.*, 2014). On one hand, martian igneous rocks might be expected to contain far less organic material than a sedimentary rock bearing the remains of abundant biota. On Earth, igneous rocks commonly contain <0.01% organic carbon (primarily as graphite), whereas organic-rich sedimentary rocks can contain 10% or more TOC. Altered igneous rocks are also reasonable candidates as they might have partially altered minerals that might have served as sources of redox energy. On the other hand, martian meteorites arriving on Earth were likely blasted into space from well below the martian surface, where any organics present would have been shielded from the highly oxidizing and radiolytic surface environment. While it is true that near-surface exposure to cosmic radiation likely degrades organic molecules, recent work on Curiosity shows that at least some locations on Mars are eroding fairly rapidly (Farley *et al.*, 2014). Such locations are likely targets for sample collection.

Amino acids constitute the best studied, and among the most interesting, classes of molecules in martian meteorites. Table 6 summarizes existing data on measured amino acid concentrations in seven martian meteorites; some of these data are unpublished, because of the difficulties inherent in distinguishing indigenous martian organics from terrestrial contaminants in these samples. Of these examined meteorites, RBT-04262 appears to be the least contaminated by terrestrial compounds, and thus provides the most reliable indications for what to expect. This sample contained glycine, β -alanine, and γ -aminobutyric acid at levels from 8 to 28 ng/g; other amino acids and nucleobases were below method detection limits in the low nanogram per gram range (reported by Callahan *et al.*, 2013). Other meteorites have generally yielded higher concentrations of amino acids, but again we cannot be certain that these are not terrestrial contaminants. The Antarctic meteorites ALHA 770051

and EETA 79001 have yielded similar concentrations of amino acids. Two caveats to these results are noteworthy: first, none of them is a sedimentary rock, and so might be expected to have lower amino acid contents than sedimentary rocks bearing life (although see the discussion above about surface oxidation and radiolysis); and second, these amino acids are not definitive evidence of life, as a shock-metamorphic origin has also been suggested. Collectively, these data suggest that some amino acids may be present in martian sedimentary rocks at concentrations (from RBT04262 as an example) ranging from a few to a few tens of nanograms per gram, and would be detectable above a background contamination level of <1 ng/g per compound.

Other studies of Martian meteorites have constrained reduced refractory carbon concentration in the low ppm range (Wright *et al.*, 1988, 1992; Grady *et al.*, 1997a,b, 2004; Steele *et al.*, 2012). In the Mars meteorites studied to date, this material is highly refractory and does not yield appreciable levels of extractable molecules. Further work on the Tissint meteorite has shown that -CN, -COOH, CO, CH, and C-C bonding and aliphatic and aromatic carbon are a component of this material, but the concentrations of these functional groups and the specific molecules concerned are not yet fully identified. Benzene, naphthalene, phenanthrene, benzonitrile, and chloromethane, among, other species have been identified in pyrolysis products (Steele *et al.*, 2012, 2013).

Studies by Sephton *et al.* (2002) and Jull *et al.* (2000) have also examined the molecular and carbon-isotopic composition of organics released by pyrolysis of martian meteorites. The primary structures released include aromatic rings (benzene, toluene, biphenyl, etc.) plus phenol and benzonitrile, with $\delta^{13}\text{C}$ values similar to those from carbonaceous chondrites (but also terrestrial organic matter). Although terrestrial contamination cannot be rigorously excluded, these authors argue that such compounds likely originate from aromatic, high-molecular-weight organic matter derived from meteoritic input to Mars. The concentrations of organic molecules in the pyrolysates were not quantified, but based on stated detection limits are likely >10 ng/g.

The Panel was not aware of any other reported results for measurements of individual organic compounds from martian meteorites. If we assume that martian organics derive primarily from delivery by carbonaceous meteorites (but note the contrary possibility that the amino acids have shock-metamorphic origins), then we can use the composition of the Murchison meteorite as a rough guide to their expected abundance. This analogy suggests that monocarboxylic acids should be present at roughly an order of magnitude higher abundance than amino acids, aliphatic and aromatic hydrocarbons, alcohols, aldehydes, and ketones at a similar order of magnitude abundance, and amines, purines, pyrimidines, and polypyroles at 1–2 orders of magnitude lower abundance (Table 7).

In situ measurements of martian organics are available from the Viking, Phoenix, and Curiosity missions. Both Viking and Phoenix studied loose sediment scooped from the martian regolith (expected to be highly oxidized), and reported no volatile organic compounds above detection limits of <1–10 ng/g (Biemann *et al.*, 1977). The SAM instrument on Curiosity has not yet completed its work or formally reported all the results in hand; however, recent

TABLE 6. SUMMARY OF THE AVERAGE PROCEDURAL BLANK-CORRECTED FREE (NON-HYDROLYZED) AND TOTAL (6 M HCL ACID HYDROLYZED) AMINO ACID CONCENTRATIONS (IN NG/G) IN THE HOT-WATER EXTRACTS OF THE SNC METEORITES

Amino acid	Tissint meteorite		Tissint soil		NWA 7034		RBT 04262		ALHA 77005		EETA 79001		ALH 84001		MIL 03346		Antarctic ice	
	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free	Total
D-Aspartic acid	3 ± 2	48 ± 9	105 ± 69	1648 ± 442	21 ± 5	199 ± 50	< 1	< 1	2	2	< 2	< 2	< 2	< 2	10	0.01E-3	0.01E-3	0.01E-3
L-Aspartic acid	27 ± 1	196 ± 32	1331 ± 76	4341 ± 1489	233 ± 150	531 ± 152	< 6	< 1	< 2	< 2	< 2	< 2	< 2	< 2	27	0.01E-3	0.02E-3	0.02E-3
D-Glutamic acid	1 ± 1	59 ± 29	56 ± 5	1501 ± 761	14 ± 3	500 ± 53	< 1	< 1	2	2	2	2	< 2	< 2	10	< 0.01E-3	0.01E-3	0.01E-3
L-Glutamic acid	12 ± 1	548 ± 187	630 ± 17	9485 ± 4312	157 ± 26	1,756 ± 503	< 2	< 1	10	17	< 2	< 2	< 2	< 2	84	< 0.01E-3	< 0.01E-3	< 0.01E-3
D-Serine	17 ± 5	32 ± 5	423 ± 319	1811 ± 610	149 ± 57	183 ± 26	< 1	< 2	1	2	< 2	< 2	< 2	< 2	4	< 0.01E-3	< 0.01E-3	< 0.01E-3
L-Serine	99 ± 1	273 ± 15	3771 ± 112	5841 ± 1276	871 ± 683	771 ± 373	< 19	< 2	< 2	< 2	< 2	< 2	< 2	< 2	25	0.02E-3	0.05E-3	0.05E-3
Glycine	97 ± 11	432 ± 120	3,311 ± 695	10,524 ± 597	498 ± 410	1,572 ± 500	28 ± 9	22 ± 3	2	74	2	74	60	60	256	0.02E-3	0.03E-3	0.03E-3
β -Alanine	7 ± 1	42 ± 16	382 ± 110	1697 ± 482	45 ± 1	305 ± 156	8 ± 1	64 ± 17	11	14	14	14	50	50	16	< 0.01E-3	< 0.01E-3	< 0.01E-3
γ -Amino- <i>n</i> -butyric acid	8 ± 1	58 ± 8	290 ± 69	1812 ± 680	43 ± 13	109 ± 90	14 ± 2	133 ± 12	51	53	53	53	178	18	18	0.01E-3	< 0.01E-3	< 0.01E-3
D-Alanine	8 ± 1	28 ± 10	162 ± 2	1244 ± 444	73 ± 48	168 ± 46	< 2	< 6	< 2	1	< 2	< 2	< 2	< 2	3	0.01E-3	0.01E-3	0.01E-3
L-Alanine	41 ± 1	167 ± 29	1459 ± 85	6117 ± 1600	347 ± 148	910 ± 224	< 7	4 ± 1	< 2	< 2	< 2	< 2	8	11	11	0.01E-3	0.01E-3	0.01E-3
D- β -Amino- <i>n</i> -butyric acid	< 3	< 5	< 60	< 60	< 2	< 2	< 4	< 4	< 2	< 2	< 2	< 2	< 2	< 2	< 2	0.01E-3	0.05E-3	0.05E-3
L- β -Amino- <i>n</i> -butyric acid	< 3	< 5	< 60	< 60	< 2	< 2	< 4	< 4	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 0.01E-3	< 0.01E-3	< 0.01E-3
α -Amino-isobutyric acid	< 3	< 6	< 80	< 80	< 1	< 2	< 4	< 5	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 0.01E-3	< 0.01E-3	< 0.01E-3
DL- β -Amino-isobutyric acid	< 5	< 5	< 80	< 80	< 5	< 5	< 5	< 5	ND	ND	ND	ND	ND	ND	ND	< 0.01E-3	< 0.01E-3	< 0.01E-3
DL- α -Amino- <i>n</i> -butyric acid	< 4	< 5	< 90	< 90	< 8	< 4	< 5	< 5	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 0.01E-3	< 0.01E-3	< 0.01E-3
DL-Isovaline	< 4	< 8	< 95	< 95	< 2	< 3	< 5	< 8	< 2	< 2	< 2	< 2	1	1	1	< 0.01E-3	< 0.01E-3	< 0.01E-3
3-Amino-2,2-dimethylpropanoic acid	< 1	14	< 48	76	< 1	11	< 1	< 1	ND	ND	ND	ND	ND	ND	ND	< 0.01E-3	< 0.01E-3	< 0.01E-3
5-Aminopentanoic acid	1	7	41	770	17	36	1	4	2	2	1	1	14	1	1	< 0.01E-3	< 0.01E-3	< 0.01E-3
ε -Amino- <i>n</i> -hexanoic acid ^a	5 ± 4	25 ± 7	151 ± 16	2146 ± 771	246 ± 146	690 ± 527	53 ± 17	1,200 ± 780	244	153	153	153	1,439	126	126	< 0.01E-3	< 0.01E-3	< 0.01E-3
D-Valine	2 ± 2	10 ± 7	99 ± 19	143 ± 113	16 ± 19	22 ± 20	< 1	< 1	< 2	< 2	< 2	< 2	< 2	< 2	< 2	< 0.01E-3	< 0.01E-3	< 0.01E-3
L-Valine	13 ± 1	121 ± 16	280 ± 272	3558 ± 1200	230 ± 74	400 ± 150	< 2	< 1	< 2	< 2	7	7	3	23	23	0.01E-3	< 0.01E-3	< 0.01E-3

Data are from Tissint and Moroccan soil (Steele *et al.*, 2014), NWA 7034 (Steele *et al.*, 2013), RBT 04262 (Callahan *et al.*, 2013), ALHA77005, EETA79001, ALH 84001, MIL 03346 (Callahan, unpublished data), and Graves Nunataks Antarctic ice (Burton *et al.*, 2012). All values are reported in parts per billion (ppb) on a bulk sample basis except where noted. Extracts were analyzed by OPA/NAC derivatization and high-performance liquid chromatography separation with UV fluorescence detection and by LC-ToF-MS at NASA GSFC. The uncertainties are based on the standard deviation of the average value of two separate measurements.

^aAlso known as *ε*-amino-*n*-caproic acid (EACA), which is also the hydrolysis product of Nylon-6.

ND, not determined.

TABLE 7. DISTRIBUTION OF CARBON IN THE MURCHISON CM2 METEORITE

Substance	Abundance
Insoluble carbonaceous phase	1.3–1.8%
Carbonate and CO ₂	0.1–0.5%
Aliphatic hydrocarbons	12–35 ppm
Aromatic hydrocarbons	15–28 ppm
Monocarboxylic acids (C2–C8)	~170 ppm
Hydroxy acids (C2–C5)	~6 ppm
Amino acids	10–20 ppm
Alcohols (C1–C4)	~6 ppm
Aldehydes (C2–C4)	~6 ppm
Ketones (C3–C5)	~10 ppm
Ureas	~20 ppm
Amines (C1–C4)	~2 ppm
Pyridines and quinolones	0.04–0.4 ppm
Pyrimidines	~0.05 ppm
Purines	~1 ppm
Polypyrroles	< 1 ppm
Sum	1.43–2.35%
Total carbon	2.0–2.5%

Data are from Wood and Chang (1985) and Cronin and Chang (1993).

abstracts and papers in preparation describe the “tentative” detection of dichloropropane and chlorobenzene at levels of a few tens of nanograms per gram in a core drilled into a mudstone at Yellowknife Bay (*e.g.*, Summons *et al.*, 2014a,b). A significant caveat here is that all three missions used thermolysis and pyrolysis to volatilize organic compounds so that they are amenable to gas chromatographic and mass spectrometric analysis. A series of studies (Navarro-González *et al.*, 2006, 2010, 2011; Navarro-Gonzalez and McKay, 2011) suggested that the presence of perchlorate and other oxidants in the martian regolith renders the Viking results difficult to interpret because of the high probability of either oxidizing or chlorinating indigenous organic molecules during heating. These observations have been contested (Biemann, 2007). As a result of these complications, the Panel did not rely on the earlier non-detections of organics in Mars regolith. The more recent detections of chlorobenzene (or possibly its aromatic precursors) and other chlorohydrocarbons by MSL (*e.g.*, Freissinet *et al.*, 2014) are considered to provide likely lower limits for these compounds.

The OCP briefly considered organic concentrations in analog terrestrial rocks as another constraint on what to expect on Mars. Fine-grained sedimentary rocks that have not been oxidized or weathered constitute the type of sample that we might hope to return from Mars. On Earth, similar rocks commonly contain >0.1% (100 ppm) TOC, and contain many individual biomarkers at levels > 1 ppm. Even those sediments considered to be relatively poor in organics contain >0.01% (10 ppm) TOC (Mayer, 1994), and yield individual biomarkers at levels >10 ng/g (*e.g.*, Lipp *et al.*, 2008). Soils from the Atacama Desert are reported to have 32 ppm TOC (Navarro-González *et al.*, 2010), an order of magnitude below typical “organic poor” marine sediments. Subcritical water extraction of subsurface Atacama soils (Jungay region) followed by derivatization and capillary electrophoresis of the fluorescently labeled amines has

demonstrated individual amines and amino acids at the 50–100 ng/g level (Skelley *et al.*, 2007). The earlier MSR SSG II panel considered a more extreme example of organic-poor sediments (*i.e.*, a highly oxidized sedimentary rock that had been buried and undergone thermal maturation). Although such rocks have similar levels of TOC (approximately 0.01%), diagenesis has rendered most biomarkers into macromolecular kerogen, which is not extractable. They thus estimated expected concentrations for hydrocarbon biomarkers of 0.1–1.0 ng/g in such a rock (see Section 2.4 for details of this calculation).

There are several limitations to using such terrestrial analogs to predict concentrations of martian organics. First and foremost, there is no *a priori* reason to expect that concentrations of organics in terrestrial rocks would be indicative of those on Mars. Indeed, valid arguments can be made for their being either higher or lower than indigenous martian concentrations. For example, with lower organic input and more oxidizing subsurface conditions, martian rocks might have lower organic concentrations. If microbial activity were more limited (or absent) on Mars, residual organic concentrations might be higher. Second, it is unclear which terrestrial rocks, sediments, or soils we should choose as appropriate analogs. Even considering that oxidized rocks represent a reasonable lower bound for those found on Mars, the goal of the mission is clearly *not* to sample and return the most oxidized martian rocks. Indeed, it is unclear whether scientific goals could be met with such a rock even given zero organic contamination. The third concern expressed by the OCP is that hydrocarbons (the dominant biomarkers in thermally mature terrestrial rocks, and those considered primarily by the MSR SSG II report) may not be the class of organics that are most abundant or interesting on Mars. With no active tectonics to deeply bury sediments under reducing conditions, biomolecules (or even meteoritic organic compounds) might be transformed to more oxidized species rather than more reduced ones. In summary, consideration of terrestrial analog rocks indicates that organic concentrations in martian rocks might span a huge range around those directly measured in meteorites. We therefore conclude that this line of argument provides little firm footing on which to construct quantitative limits.

A final constraint on expected concentrations *in the absence of martian biota*, previously considered by the OCSSG report and by Benner *et al.* (2000), can be derived from estimated rates of delivery of organic carbon to the surface of Mars by meteorites. Meteorites deposit an estimated 2.4×10^8 g/year of organic carbon to the martian surface (Flynn, 1996). If allowed to accumulate over 3 billion years, and given a Martian surface area of 3.6×10^{13} m², this would result in 20 kg/m² of organic carbon. Assuming a mixing depth of 1 m and rock density of 4 g/mL results in a predicted TOC concentration of 5 mg/g. A much more conservative mixing depth of 100 m would lower this to 50 µg/g. If we presume that this organic carbon has a molecular makeup similar to that of Murchison (see Table 7), where functional classes of molecules represent approximately 0.05% of TOC, we predict approximately 2.5 µg/g of each class of organics (1 m mixing depth). Further assuming that each class comprises 10–100 compounds, this yields a final prediction of approximately 20–200 ng/g per compound (or 0.2–2 ng/g for the 100-m mixing depth). These estimates span

the measured concentrations described above (calculations presented here follow those in Benner *et al.*, 2000).

A significant complication is that much of this meteoritic carbon is likely to be oxidized on the martian surface. Benner *et al.* (2000) considered that many of the likely oxidation products are organic molecules that would be metastable under martian conditions (*e.g.*, benzenecarboxylic acids). Working from the assumption that meteoritic TOC would be converted to such molecules with an efficiency of approximately 10%, they predicted (using the same delivery rate and mixing depths as above) an accumulation of 5–500 $\mu\text{g/g}$ of benzenecarboxylic acids. The concentrations of individual species were not predicted, but again assuming 10–100 major compounds would yield a range of estimated concentrations of 50 ng/g–50 $\mu\text{g/g}$ per compound. These values are higher than those estimated for individual molecules in the preceding paragraph due to the assumption that meteoritic TOC (as opposed to just extractable carbon) is converted into measurable molecules. Moreover, laboratory analog experiments show that heating functionalized aromatics such as benzenecarboxylic acids in the presence of perchlorate generates chlorobenzene as identified by Curiosity (Miller *et al.*, 2013).

4.2.2.2. What concentrations can we measure? Instrument detection limits provide a useful lower bound for setting organic contamination requirements in two ways. First, it is not feasible to set requirements that are below the detection limit of any analytical method, because there would be no way of verifying that requirements have been met. Second, there would be little practical benefit in protecting analyte concentrations that are themselves too low to measure, although here one needs to account for the inevitable improvements in sensitivity that would exist by the time samples are returned. Regardless, it is not necessarily true that contamination limits need to be lower than detection limits to protect all measurements (see Section 4.1.2). Another key aspect is that the ability to characterize contamination levels (*e.g.*, their reproducibility and chemical nature) might improve in the future.

As described in Section 3, there is a huge diversity of analytical techniques that could be brought to bear on returned martian samples, each differing in their sensitivity towards analytes (limits of detection) and their selectivity (ability to measure a wide range of compounds). Based on the general trade-off between selectivity and sensitivity, we argue that it is not practical to consider the detection limits of all possible analytical techniques, and instead focus on those with the broadest analytical window (least selective) that would be used for initial assessment of the types and concentrations of organics present in samples (survey measurements). As described above, detection limits for the most sensitive of these methods are typically in the range of 0.1–10 ng/g. We thus adopt 1 ng/g as a representative value.

A limit of 1 ng/g for individual organic compounds of primary concern appears to strike a good compromise between protecting the survey measurements that would undoubtedly be conducted on returned samples, while still being detectable by more targeted measurements for validation of spacecraft contamination levels. The primary perceived downside to this strategy is that a 1 ng/g limit would not fully protect even the most sensitive techniques

existing today, let alone those that might exist 20 years in the future. Clearly, lower contamination limits would not be “wasted” in the sense that they would enable even more sensitive measurements to be made. If lower contamination levels (for example, down to 0.01 ng/g) can be achieved at reasonable expense, they would be scientifically valuable. On the other hand, the few concrete measurements of martian samples that do exist (described above) do not indicate that such stringent limits would be absolutely required to meet mission scientific objectives.

4.2.2.3. What level of cleanliness can we achieve? The third criterion considered by the Panel is what levels of background contamination can be achieved using current technology. At the level of individual compounds, this is a difficult issue to grapple with for a number of reasons. First, contamination varies widely in space and time, as a function of analytical conditions, target analytes, sample matrices, etc. Compiling estimates for every compound of interest, under even a few sets of conditions and samples, is prohibitive. Second, eliminating contamination is difficult and time-consuming, and so typically is driven only to the levels that are needed, rather than those that are technologically possible. Sub-nanogram per gram levels of organic analysis are uncommon in terrestrial geologic samples, so similar levels of cleanliness are seldom needed. Third, quantitative background concentrations are not often reported, rather simply that they are below the levels of detected analytes. Fourth, methods of reducing contamination are often specific to the particular analytes of interest, and so it is unclear whether it is possible to simultaneously achieve reported limits for all analytes of interest.

With these caveats, we can use the minimum levels of detection reported for various analytes in geologic samples as a maximum limit for the blank concentrations that must have been achieved by those studies. Amino acids have been measured in martian meteorites at levels down to 1 ng/g (Callahan *et al.*, 2013), fatty acids in deep-subsurface rocks have been measured down to 10 pg/g (Onstott *et al.*, 2003), DNA has been measured down to 10 fg/g by fluorescence detection (Zhao *et al.*, 2003), and PAHs have been measured down to 10 fg/mL by laser-induced fluorescence (Yan *et al.*, 1995). It thus appears likely that most, if not all, measurable organic compounds can readily be reduced to at least < 1 ng/g, and in many cases to orders of magnitude below that. We thus foresee no significant difficulties in achieving the levels of cleanliness indicated in preceding sections, although it is conceivable that achieving such limits simultaneously for all Tier-I compounds may be more difficult.

4.2.3. Conclusions for specific compound levels. Based on the evidence discussed above, our best estimate for concentrations of the most abundant organic molecules of interest (*i.e.*, Tier-I compounds) in returned martian rocks is in the range of 1–100 ng/g. We thus believe that these compounds would likely be measurable above background contamination comprising < 1 ng/g per compound. Such background levels should be readily achievable given current technology, and would be at the low end of what is measurable by current survey analytical techniques, thus protecting their role in initial characterization of returned samples. We therefore propose a maximum limit for Tier-I compounds, on a mass/mass basis in

returned samples, of 1 ng/g (*i.e.*, < 1 ppb). It is of course possible that analyte concentrations in the returned samples may turn out to be lower than expected, and so the odds of scientific success would be improved by still lower contamination limits. Moreover, lower background levels would permit more accurate and precise measurements at any concentration. Nevertheless, given currently available evidence, it is hard to build a case that contamination limits substantially lower than this would be *required* to meet either scientific or PP objectives.

Compounds in Tier II comprise all other organic molecules, of significant potential interest, but of lesser priority. It is unrealistic to require a spacecraft development team to monitor *all* organic molecules, and OCP's intent is for Mars 2020 to propose an implementation plan based on one or two broad-based analytic methods that are capable of scanning a wide spectrum of organic molecules. Although we do not expect Tier-II compounds to be present at higher level than those in Tier I, the Panel assigned a lesser importance to protecting the measurements of these compounds, because they are likely not required to meet the mission scientific objectives. We therefore propose a 10-fold higher limit for these compounds of 10 ng/g per compound. While it is true that such a limit could compromise the measurement of Tier-II compounds present at low nanogram per gram levels, it is unlikely to significantly interfere with the measurement of Tier-I compounds, our highest priority. Moreover, given the TOC limits we propose (below), it is impossible for more than a few Tier-II compounds to approach this limit, implying that many other compounds would still be measurable at much lower levels.

A frequent point of discussion for the OCP was "What happens to compounds that are less abundant in the returned samples than those discussed above?" Certainly it is inevitable that many compounds would be present at < 1.0 ng/g. However, setting a contamination limit of 1 (or 10) ng/g does not imply that every compound in Tier I (II) would be present at that level. Rather, sampling surfaces would be cleaned until the most abundant contaminant meets that level, and most other compounds would then be present at much lower levels. The Panel thus believes that this strategy represents a sensible compromise, providing reasonably achievable goals while at the same time ensuring that the vast majority (though not necessarily all) analytes of interest would be measurable.

Major Finding #13: We propose the following limits for organic contamination of geological samples by specific compounds: 1 ng/g for Tier-I compounds deemed as essential analytes for mission success and 10 ng/g for Tier-II compounds (all others).

4.3. Considerations related to TOC

A limit for TOC contamination can serve two purposes. First, it serves to limit background levels that must underlie measurements of TOC (whether of concentration, elemental or isotopic composition, molecular weight, etc.) in returned samples. Second, it serves as a simple way to effectively limit contamination at the molecular level by all possible compounds, without the analytical effort required to quantify them individually. Given that TOC is almost universally more abundant than individual compounds by orders of magnitude, the second consideration serves as the more

stringent constraint. The OCP therefore focused primarily on setting TOC limits that would achieve effective protection of measurements at the molecular level. Previous missions seem to have adopted a similar philosophy in setting TOC limits, particularly given that levels for individual molecules were rarely established. In this context it is important to remember that our proposed TOC limit is not an end unto itself, but rather must be consistent with the molecular limits proposed above. Relaxing requirements for individual molecules would likely lead us to recommend more stringent limits on TOC.

4.3.1. Allowable levels of contamination. As for individual contaminants, the Panel considered three lines of evidence in choosing appropriate limits. The discussion below deals exclusively with data for TOC, primarily the background levels required for protecting TOC measurements. We return to the question of protecting measurements of individual molecules in Section 4.4.

4.3.1.1. *What analyte concentrations do we expect?* The most recent and complete study of TOC in martian meteorites reported levels ranging from 4 to 26 $\mu\text{g/g}$ (ppm) in all seven samples subjected to this analysis (Steele *et al.*, 2012). To avoid analyzing terrestrial contaminants, the authors first heated samples to 600°C to drive off volatile and semi-volatile compounds. The reported concentrations thus represent a minimum limit for the true total organic load of the samples prior to landing on Earth. Further analysis by Raman spectroscopy and laser-desorption mass spectrometry indicated discrete blebs of macromolecular carbon trapped within mineral grains, consistent with an indigenous martian origin. Analyses of ^{13}C and ^{14}C in the samples provided further support for a dominantly martian origin. Similar, albeit non-spatially resolved, data have previously been obtained by Grady *et al.* (2004). Given that these samples are all martian basalts, it appears quite likely that most unaltered martian rocks contain a background level of at least 1 $\mu\text{g/g}$ (ppm) of TOC. Whether or not sedimentary rocks and oxidized regolith contain more or less TOC is subject to assumptions about the relative importance of biotic and meteoritic inputs versus oxidative loss (see above), and appears unknowable at the current time.

Finding #14: Although TOC from Martian igneous minerals can reach 20 $\mu\text{g/g}$ (ppm), and are > 4 $\mu\text{g/g}$ in all samples measured to date, the concentration in non-igneous samples is currently unknown, and may be much lower.

4.3.1.2. *What concentrations can we measure?* TOC is an operationally defined fraction subject to all the ambiguities that accompany the definition of "organic carbon." Consequently, analytical methods for measuring TOC concentration (Table 8) do not all report the same value from the same sample. For example, methods based on oxidative combustion at temperatures > 800°C include all carbon that can be oxidized to CO_2 . Such methods commonly have detection limits near 1 $\mu\text{g/g}$ without concentrating samples, although this appears to be limited more by terrestrial blanks than by instrument sensitivity. Some methods based on

TABLE 8. COMPILATION OF ANALYTICAL METHODS, AND REPRESENTATIVE SENSITIVITY, POTENTIALLY SUITABLE FOR MEASURING TOC IN SAMPLES AND/OR ON SPACECRAFT SURFACES

	Method	Sensitivity	Sampling/Form*	Comments
Vibrational Spectroscopy	FTIR-DRIFT	<1 ng/cm ² (from 100 cm ²)	Witness plate or solvent extract**	Provides broad range of chemical functional groups and/or identification. Applied to numerous spacecraft missions, detects common AC and spacecraft molecular contamination. Large spacecraft database.
	FTIR-GATR	Sub-monolayer 0.5 ng/cm ²	Witness plate or solvent extract	Provides chemical functional groups and identification, detects common AC. Rapid.
	FTIR-Microscopy	Sub-nanogram particles	Specialized witness plate	Requires specialized witness plate or particle sampling. Rapid
	Raman-Microprobe	Sub-nanogram particles	Specialized witness plate	Requires specialized witness plate or particle sampling. Rapid
Mass Spectrometry (MS)	GC-MS	<0.1 ng/cm ² (from 100 cm ²)	Witness plate or solvent extract	Identification of components in a complex mixture, non-volatile components not detected, detects common AC.
	Pyrolysis GC-MS	<0.1 ng/cm ² (from 100 cm ²)	Witness plate or solvent extract	Detects non-volatile components not detected in GC/MS, can run in series with GC/MS.
	DART-MS	<0.001 ng/cm ² (from 100 cm ²)	Witness plate or solvent extract	Identification of components in a complex mixture, MW>1000 amu requires pyrolysis, detects common AC, very sensitive, rapid.
	LC-MS	<0.1 ng/cm ² (from 100 cm ²)	Witness plate or solvent extract	Identification of components in a complex mixture, somewhat complex procedures and method development, particularly well-suited for some biological analytes.
	LD-MS	<1 ng/cm ²	Witness plate or solvent extract	Identification of components in a complex mixture, suited for high MW bio-analytes, complex procedures and method development, expensive instrumentation.
	SIMS	Sub-monolayer	Witness plate	Quantitation difficult, very sensitive, limited molecular identification or organics, very sensitive, detects common AC, complex, expensive instrumentation.
Other	XPS/Auger	Sub-monolayer	Witness plate	Sensitive, elemental information, limited molecular identification, detects common AC, complex, expensive instrumentation.
	TOC Instruments (Pyrolysis and electrochemical)	~3 ng/cm ²	Witness plate	No chemical information, no identification, does not quantify incombustible components.

*It should be noted that all methods require specialized hardware sampling and/or witness plates.

**Solvent extracts may use a surface rinse or specialized solvent swabs of hardware surfaces.

GATR, grazing angle attenuated total reflection (IR); MW, molecular weight.

vibrational spectroscopy (FTIR, Raman, etc.) are typically more sensitive (down to 1 ng/g levels), but may not detect all carbon species; for example, FTIR cannot detect graphitic or amorphous macromolecular carbon. On the other hand, surfaces can be mapped using Raman spectroscopy to survey for minute concentrations of these species. However, these techniques require delicate surface preparation and are susceptible to topographic and matrix effects. With so many analytical dependencies, they are generally not quantitative.

Mass spectrometric methods for analyzing TOC in solvent extracts are equally sensitive, but have more restrictive analytical windows and would likely not include macromolecular carbon. Other techniques, such as secondary ionization mass spectrometry (SIMS) and x-ray photoelectron spectroscopy (XPS) are highly sensitive, but are more complex and ex-

pensive to implement. A second complication is that some TOC methods are amenable only to analyses of bulk sample materials (oxidative combustion), whereas others are amenable only to analyses of surfaces (SIMS, FTIR, Raman, XPS). Some sample-based measurements can be applied to spacecraft surfaces by means of solvent rinses or swabs.

Beyond simple measurements of TOC concentration, it is likely that scientists would be interested in assaying the elemental and isotopic composition, average molecular weight, aromaticity, optical activity, ion-exchange capacity, surface-area loading, and other characteristics of total organic matter in returned samples. Such measurements all use specialized analytical techniques, only some of which overlap with those described in Section 3. Nevertheless, it is the Panel's belief that all such measurements would be less

sensitive than those used to measure simple TOC concentrations, and so they are not considered further here.

The OCP discussed these analytical issues in detail, but ultimately decided that it was beyond our purview to provide a detailed intercomparison of methods, or to propose specific methods for monitoring TOC. For the purpose of setting minimum contamination limits, we point out that several surface-based techniques can routinely achieve detection limits of $<1 \text{ ng/cm}^2$, and so could be employed to verify contamination limits on spacecraft surfaces down to this level. Translating this into a bulk contamination level for samples requires consideration of both transfer efficiency and sample-contact surface (see below), but leads to plausible levels of 2–20 ng/g TOC. To our knowledge, no bulk TOC analyses of terrestrial geologic samples have yet reported detection limits this low. On the other hand, no terrestrial geologic materials have such low levels of TOC, so these detection limits have presumably not been needed. It is our belief that if a martian sample were returned that contained only 2–20 ng/g TOC, a suitable analytical method for quantifying this level of carbon could be readily developed.

4.3.1.3. What level of cleanliness can we achieve?

There was a very substantial debate among OCP panelists as to what are the lowest levels of TOC contamination that are readily achievable. In considering this issue, a key concept is to distinguish between what is possible on spacecraft surfaces, and what is achievable in returned samples. The former can be readily reduced to arguments about nanograms per unit area, but the latter requires additional understanding of contaminant contributions from the entire sampling train, and potentially the entire landed spacecraft. Hardware geometry and mode of operation, as well as surface cleanliness, become relevant. We consider the issue of cleaning surfaces first and that of the integrated sample contamination second.

Methods for cleaning spacecraft surfaces vary widely, and with highly variable results. As an optimal solution, the Panel agreed that extended (hours) heating to $>500^\circ\text{C}$ in an oxidizing atmosphere (commonly air) typically renders a surface free of organic carbon at or below picograms per gram (ppt) levels. This procedure is commonly used in most organic geochemical laboratories today. The question then becomes how rapidly the surface becomes recontaminated with organic carbon. Earth's atmosphere contains an appreciable amount of organic carbon, both in the form of volatile organic compounds and as organic particulates. The latter are readily filtered out of clean-room air, but the former are harder to control to extremely low levels. Thus a bare metal surface exposed to even "clean" air will quickly (within minutes/hours) acquire a layer of AC, typically approximately $20\text{--}100 \text{ ng/cm}^2$. The rate of recontamination is highly dependent upon the type of organic carbon present, their partial pressures within the environment, and the temperature differential between the environment and the spacecraft surfaces. Deposition rates differing by orders of magnitude can be achieved by covering cleaned parts with a clean impermeable wrapping such as aluminum foil. The phenomenon of accretion of contaminants is well documented in the literature (*e.g.*, Siegbahn *et al.*, 1967; Swift, 1982; Barr and Seal, 1995; Piao and McIntyre, 2002).

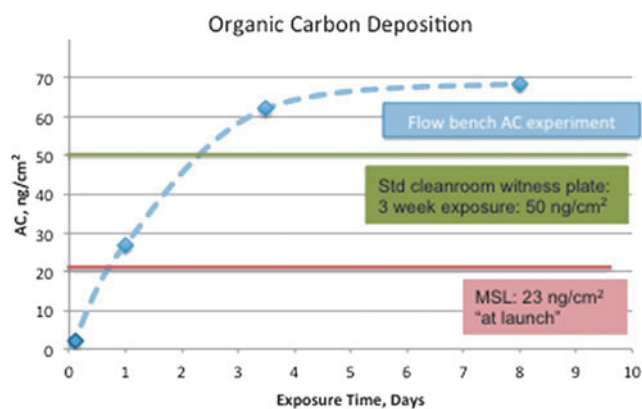


FIG. 11. JPL test data suggest an asymptotic time dependence for the formation of an AC film on precleaned metal surfaces. Rate and amount of formation are highly variable, and depending on precursor concentrations, substrate, configurations, and environmental conditions. The quantification of the y-axis is TOC. Std, standard.

Studies indicate that this layer asymptotically approaches a stable, approximately monolayer equivalent film (Fig. 11), with the equilibrium TOC content of this film depending both on surface composition and on volatile organic concentrations, among other factors. Typical mean deposition rates for clean-room environments are reputed to be near $0.15 \text{ ng/cm}^2/\text{hr}$ ($0.1 \text{ mg/ft}^2/\text{month}$), suggesting that a stable AC film typically requires 6–9 days to develop. Limiting contact of a metal surface to air shortly after baking should thus provide a straightforward and efficient way of achieving very low (ppm) TOC levels. Although this can be done with minimal effort on Earth, "unwrapping" the protected parts for use once on Mars would presumably introduce additional engineering risks. Although lower levels of organic contamination could be achieved by combusting (at $>500^\circ\text{C}$ in an oxidizing atmosphere) and then isolating the entire sampling hardware chain, hermetically sealing all elements of the sample chain has serious implications for mission failure modes (and opportunities to compromise science).

4.3.1.3.1. Contamination pathways. In the case of MSL, the vectors for the transfer of Earth-sourced organic contamination to rock/soil samples have been interpreted by the MSL project engineers to include the following (Fig. 12):

1. *Direct contact:* Direct contact of Mars sample material with sampling hardware is thought to be the largest contributor to sample C contamination. Based on contamination modeling, direct transfer from spacecraft contact surfaces has been interpreted to be quantitatively the most significant component for both MSL and OSIRIS-REx (Harstad and Bellan, 2006; Blakkolb *et al.*, 2008; ten Kate *et al.*, 2008; Anderson *et al.*, 2012; Dworkin, unpublished data, 2014).
2. *Particle transport:* Dislodgment of particulate (potentially microbe-laden) contamination from the exterior of the Rover by saltation is not believed to be a significant source of TOC in samples. Beaudet (2000) calculated particle dislodgement rates, not taking into account particle adhesion. Harstad and Bellan (2006)

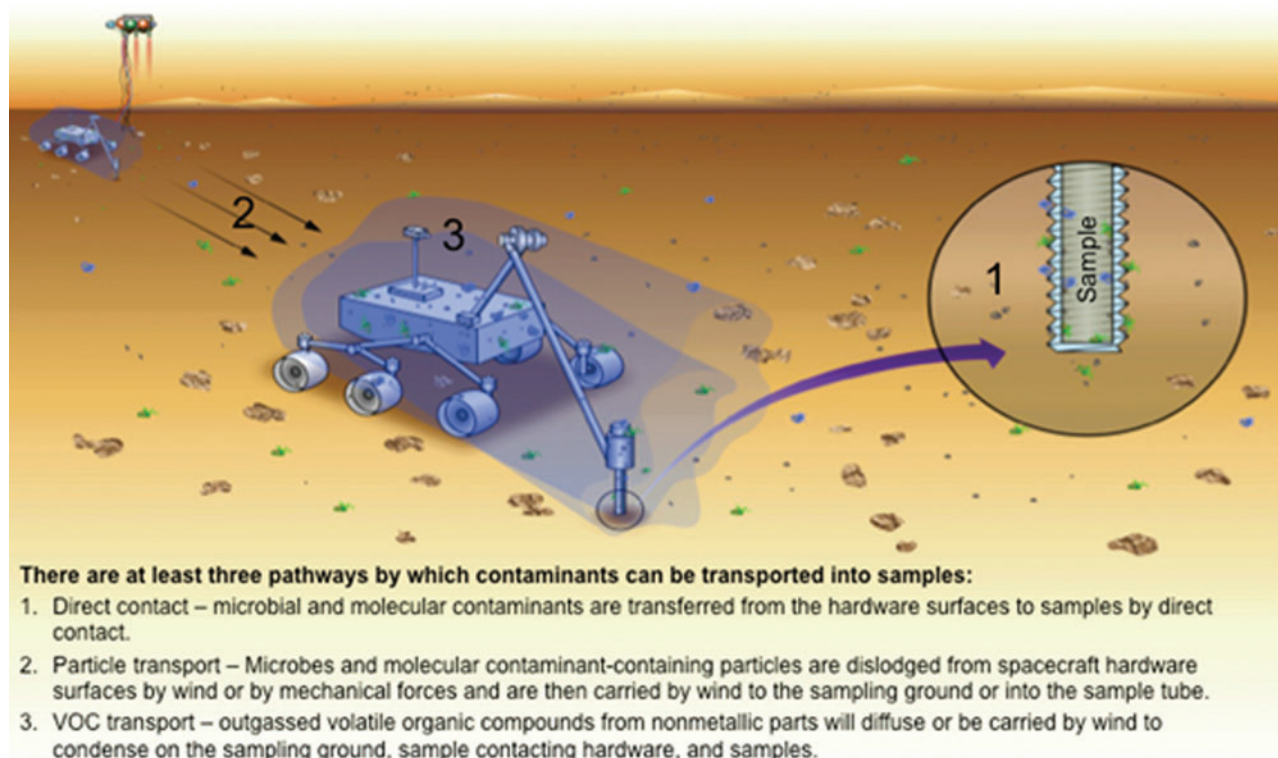


FIG. 12. Graphic representation of three important pathways for contamination transport from spacecraft to samples. VOC, volatile organic compound.

performed calculations that included adhesion forces and concluded the probability of particle removal “...is estimated as corresponding to an adhesion half-life of $O(10^4)$ years, and is thus not important.”

3. *Outgassing from rover hardware:* Engineering contamination transport calculations [using MSL transport models and source rates (Blakkolb *et al.*, 2008; ten Kate *et al.*, 2008)] show that outgassing from Rover hardware contributes less than approximately 1 ng/g TOC during the sample acquisition process (Blakkolb *et al.*, 2008).

A comparable analysis for Mars 2020 has not yet been carried out. For the purpose of this report, we confine our analysis to that of direct contaminant transfer from hardware surfaces to samples, while noting that other transport vectors need to be rigorously evaluated by the Mars 2020 Engineering Team using deterministic and probabilistic methods (see, for example, Hudson *et al.*, 2010). This reduces the problem to two variables: the hardware surface area contacting samples, and the efficiency with which the contamination is transferred.

A comparable analysis for Mars 2020 has not yet been carried out. For the purpose of this report, we confine our analysis to that of direct contaminant transfer from hardware surfaces to samples, while noting that other transport vectors need to be rigorously evaluated by the Mars 2020 Engineering Team. This reduces the problem to two variables: the hardware surface area contacting samples, and the efficiency with which the contamination is transferred.

However, changes in payload, sampling philosophy, and cache placement dictate that the Mars 2020 Project Team will need to undertake contamination transport analyses and models of transport modes that are specific to the Mars 2020 system recontamination profile. Factors influencing the induced contamination environment include, but are not limited to, the relative configuration of the cache and the instrument payload, especially potential outgassing sources from the instruments that have heaters on them, and the fact that the dilution cleaning strategy cannot be applied to Mars 2020.

Because the baseline configuration of the sample contact surfaces for Mars 2020 is very different from those of MSL, the dilution cleaning process appears not to be available to Mars 2020. However, it is important to note that in comparison with Mars 2020, MSL has far higher sample contact surface area, and far lower sample masses, so the effects of surface contamination are greatly magnified.

Finding #15: In order to achieve contamination levels for sample contact surfaces lower than 20 ng/cm^2 , a more effective strategy for avoiding recontamination after initial cleaning than that used by MSL would need to be implemented.

4.3.1.4. Contamination transfer from sample-contacting surfaces. Hardware surfaces relevant to cached samples comprise two main components: the sample (cache) container itself, and the sample collection (drilling) and

transfer apparatus. Sample containers are not intended to be re-used, so we need consider only their surface area. In contrast, current design ideas for drilling apparatus include both re-usable and single-use designs. Re-useable drill strings have the advantage of being able to undergo “dilution cleaning” [*i.e.*, dilution of terrestrial contaminants by repeated processing of martian samples (Anderson *et al.*, 2012)]. For our analysis, we considered only the most conservative case of single-use drill strings (*i.e.*, no dilution of contaminants). Several hardware designs representing a range of sample-contacting surface areas are currently under consideration by the Mars 2020 Project Team. To constrain the problem, we use 300 and 30 cm² as the respective upper and lower limits on the area of sample-contacting surfaces.

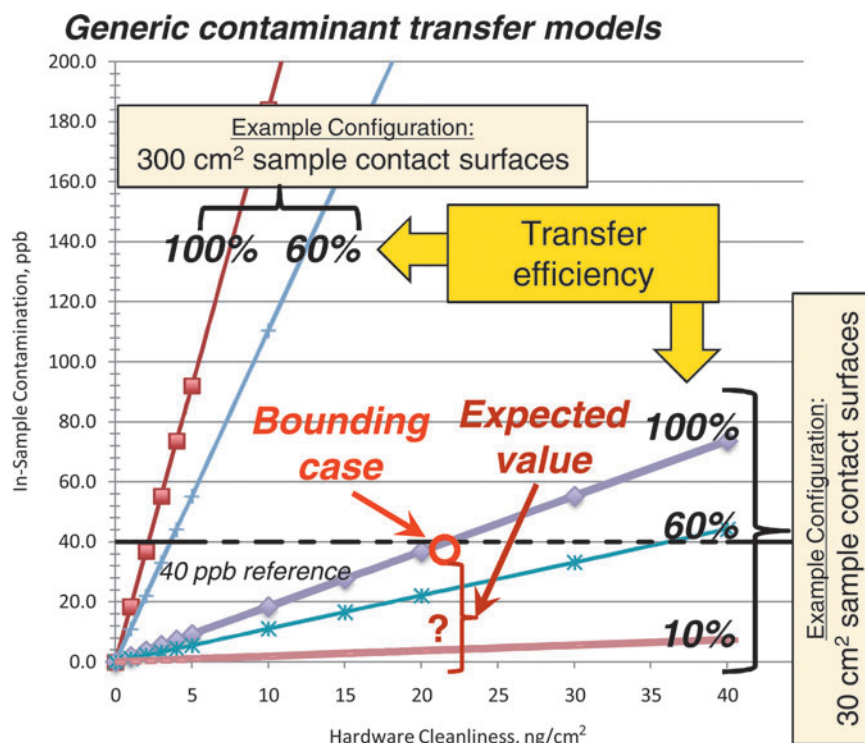
Although it is likely that most of the sample-contacting surfaces would be subject to only moderate or slight abrasion, for the purposes of our analysis we consider two cases: a non-realistic but conservative bounding case, in which 100% of the contaminants on sample contact surfaces are assumed to transfer to the samples, and a “mean expected” case, where the available experimentally determined transfer coefficients are used. If further experiments by the project on actual sampling hardware can demonstrate lower transfer efficiencies, then lowering this assumption would be reasonable.

We also assume the sample would contact the bit (a strong abrasion environment) and its sample tube (a “slight abrasion” environment), and that 25% of the sample contact surface area is bit, and 75% is tube. Such a model would imply a mean transfer efficiency of approximately 20%. This figure would drop if the bit were re-used for multiple samples due to dilution of contaminants (because a portion of the organic contaminants initially present on the bit would end up in the first sample, and be unavailable to be transferred to the second sample).

Figure 13 presents the expected level of bulk contamination in a sample (*y*-axis) given an assumed hardware surface area, transfer efficiency, and level of cleanliness (*x*-axis). For an assumed level of surface contamination of 20 ng/cm² (see above), a sample contact surface area of 300 cm² and 100% transfer efficiency results in >> 100 ng/g TOC in the sample. In contrast, given the same assumptions but with only a 30 cm² contact area, sample contamination of approximately 40 ng/g is predicted. If a 60% transfer efficiency were assumed, that level would fall to only 8 ng/g. We thus believe that TOC contamination levels in the sample of 20–40 ng/g are achievable even when sampling surfaces are exposed to air. We note that such levels would place significant constraints on the viability of some hardware designs with large surface areas:

$$ppb = \frac{\text{hardware cleanliness level (ng/cm}^2\text{)} \times \text{surface area of the sample that contacts hardware (cm}^2\text{)} \times \text{transfer efficiency}}{\text{mass of sample core (g)}}$$

FIG. 13. Translation from sample-contacting surface values to sample values, assuming sample mass = 16 g. Contaminant contact transfer efficiency is dependent on sample-hardware configuration; a range of 10–100% is presented to illustrate the proportional dependence of in-sample contamination to this parameter.



Finding #16: In the case of a system with sample contact surfaces of 30 cm², and contaminated with 20 ng/cm² organic carbon, direct transfer could result in a theoretical maximum of 40 ppb organic contaminants on collected samples assuming 100% transfer efficiency. Actual concentrations in samples could be either higher or lower than this, depending on actual transfer efficiencies and the importance of other contaminant transfer pathways.

4.3.2. **Conclusions for TOC levels.** Given expected TOC concentrations of approximately 10 µg/g in at least some classes of martian samples, TOC contamination levels of <100 ng/g would likely be sufficient to make meaningful measurements of TOC returned samples (this would result in an S/N ratio of 100:1). Such a level of contamination would be substantially better than was achieved for Apollo and most subsequent missions. However, some martian samples likely have lower organic content than this, and the Panel unanimously agreed that setting lower TOC limits would be very beneficial. This would also be advantageous for the reduction in numbers and levels of individual organic contaminants, and for the decreased probability of significant interferences with scientific measurements. Moreover, to the extent that concentrations of individual contaminants can be sufficiently controlled via TOC limits, the analytical burden of verifying individual contaminant limits can be lessened. On the other hand, the OCP did not have enough data to understand the costs of proposing lower TOC limits. We thus struggled significantly to reach consensus on the appropriate level to recommend.

Our compromise proposal for TOC limits is 40 ng/g (ppb) in the returned samples. If the respective Tier-I and -II limits of <1 and <10 ng/g are maintained, this should be adequate to both protect measurements of TOC and prevent an excess number of individual contaminant compounds. For example, no more than four Tier-II compounds would be allowed at levels approaching 10 ng/g. At the same time, if lower TOC limits can be achieved with reasonable cost and effort, this would be substantively beneficial to returned sample science. If it were possible to return samples with TOC lowered to <10 ng/g, then the Tier-II limits would be irrelevant. If TOC could be further lowered to <1 ng/g, then Tier-I limits would also be unnecessary. This would clearly be the best outcome for the eventual returned sample science that would be done. Although, as described, it does not appear necessary to meet returned sample scientific goals, it is unclear to OCP whether it is necessary to meet PP goals.

Finding #17: We propose a limit of <40 ng/g (ppb) for TOC contamination in the returned samples.

4.4. Considerations related to particulate organic matter

4.4.1. **Introduction.** The panel was asked to consider the significance of contamination by terrestrial organic particles in samples that may potentially be returned to Earth. Organic particulates may include microbial cells (living and/or

dead), cellular debris, spores, and aggregated organic material of either biological or non-biological origin. If organic matter is in particulate form, there can be elevated potential for it being misinterpreted as forms of martian life in returned samples. Thus, Earth-sourced particulate contaminants can be very significant to both science and PP.

An essential point is whether or not the hypothesis for the detection of life on Mars incorporates the assumption that Mars life can be recognized by its differences from Earth life. We know from biogeographic studies of microbial genomes here on Earth that closely related organisms inhabiting isolated environments display significant genetic differences within a few hundreds to thousands of generations (Barrick *et al.*, 2009). There is no reason to believe that life on the Earth and putative life Mars have been continuously sharing genetic information and co-evolving. Thus, it is highly unlikely that we would find very closely related organisms on both planets. A primary detection strategy for martian organisms would therefore be to look for something that is different from the life we know here on Earth. An implication of this strategy is that terrestrial microbial contaminants could be recognized—organisms on the samples that demonstrate genetic similarity to those here on Earth would be interpreted as round-trippers (Pace, 1997; Philippe *et al.*, 2013; Rinke *et al.*, 2013). This conclusion would be especially firm if the organism in the returned sample is indistinguishable from species known to inhabit the spacecraft assembly facility, or the microbiomes of humans or domestic animals (*e.g.*, Lax *et al.*, 2014).

However, the above argument has a probabilistic dimension to it, and a key phrase in the preceding paragraph is “*highly unlikely*.” This may be a crucial point of distinction between the science and PP interpretations of returned martian samples. Every organic particle found on the surface of a returned sample would require extensive work to establish its origins and possible relationship to biology (either terrestrial or martian). Such issues make it clearly desirable to avoid *any* contaminating organic particles in the samples. However, as a practical matter this may not be possible.

Finding #18: Earth-sourced particulate organic contaminants in returned samples are potentially problematic to both science and PP interpretations due to their ability to be confused with cell-like material; however, they may also be the easiest to recognize as contamination.

4.4.2. **Analytical approaches to measuring particulates on Earth.** As part of its research, the OCP identified a number of techniques that are commonly used in research on Earth to quantify numbers of organic particles, and to distinguish biology-based particles from other organic particle contamination:

- a. **Optical imaging of particles** on smooth surfaces can be easily performed using a light microscope. It is very general and works down to the statistical sampling limit, and specific staining can be used to distinguish live from dead organisms (using fluorescent stains). Smaller particulate contamination on smooth surfaces can be carried out using electron or atomic force microscopy (practical limit at the nanometer scale). Scanning electron

microscopy (SEM), when coupled with energy-dispersive spectroscopy [EDAX (or EDX)] or XPS, can be used to differentiate organic from inorganic particulates.

Application to this study:

- Metal surfaces of Mars 2020: Effective on exposed surfaces; not applicable to tubes.
 - On Mars-sourced rock and soil samples: Sample surfaces would not be smooth.
- b. **SEM.** The total particulate load on surfaces can be estimated using SEM. EDAX or XPS can be used to distinguish elemental compositions and thus organic from inorganic. However, this requires micron-by-micron mapping of a potentially contaminated sample surface (which can be incredibly time-consuming) or the collection of surface washes.

Application to this study:

- Metal surfaces of Mars 2020: Effective on exposed surfaces; not directly applicable to tubes.
 - On Mars-sourced rock and soil samples: This approach by itself will not distinguish Mars-sourced organic particles from Earth-sourced organic particles.
- c. **Total amino acid concentration** can be sensitively measured using wipes of surfaces followed by fluorescence labeling. It is very sensitive because each amino acid is labeled with a dye and because bacterial, archaeal, and fungal cells are 55% protein by dry mass (Madigan and Martinko, 2006). This assay does work on fully hydrolyzed biopolymer and applies to protein as well with a hydrolysis step.

Application to this study:

- Metal surfaces of Mars 2020: Partially effective; swabbing tubes problematic.
 - On Mars-sourced rock and soil samples: Wipes would not work on rock and soil samples. Would not be able to distinguish whether the molecules detected are in particulate or molecular form.
- d. **Total nucleic acid fragment concentration** is another sensitive molecular method that fingerprints biological cells. This is based on the use of intercalation dyes to label double-stranded nucleic acid fragments and provide picogram sensitivity; however, this approach does not work on fully degraded polymer. DNA is only 1% of the cell mass, and the dyes label every 5–10 base pairs, so this approach is about 1,000 times less sensitive than the amino acid labeling. Ribonucleic acid (RNA) analyses can differentiate between live and dead organisms, but are more difficult to perform, particularly due to the environmental instability of RNA (which is also the reason it allows us to differentiate between live and dead organisms).

Application to this study:

- Metal surfaces of Mars 2020: Partially effective. Sampling tubes problematic.
 - On Mars-sourced rock and soil samples: Wipes would not work on rock and soil samples.
- e. **Polymerase chain reaction (PCR) and nucleic acid sequencing** is a valid approach for determining microbial contamination, in terms of both quantity and identity. It is much more specific than total nucleic acid concentration, can be extremely sensitive (1–10 bacterial equivalents), and has the advantage that it can accurately identify the

type of contamination (to the species level). Nonetheless, PCR assays on nucleic acids sampled from surfaces are challenging and can produce both false-positive and false-negative results. DNA analysis does have the advantage that samples can be stored for many years and assayed later, by PCR and/or state-of-the-art sequencing technologies that allow sequencing of individual DNA molecules.

Application to this study:

- Metal surfaces of Mars 2020: Partially effective. Sampling tubes problematic.
 - On Mars-sourced rock and soil samples: Wipes would not work on rock and soil samples.
- f. **Microbial growth.** Culture-based techniques work well for a small proportion of microorganisms that are culturable within the laboratory, and a number of these microorganisms can serve as proxies for other biocontaminants (for example, thermotolerant *E. coli* as an indicator of fecal contamination). Nonetheless, the majority of environmental microorganisms remain difficult to culture (<0.1%) using current methodologies. Molecular screening of spacecraft assembly facilities (SAFs) suggests that even fewer microorganisms from these environments have so far been captured in pure cultures (e.g., La Duc *et al.*, 2014).

Application to this study:

- Metal surfaces of Mars 2020: Partially effective—a subset of live terrestrial organisms would be detected. Sampling flight tubes problematic.
 - On Mars-sourced rock and soil samples: Partially effective—a subset of live terrestrial organisms would be detected.
- f. **Methods from the semiconductor industry.** Due to the ability of particulates to interfere with the manufacture of nanometer-scaled circuitry, there are a number of methods that have been employed within the semiconductor manufacturing industry. Measurement techniques, such as scattered laser light imaging (surfscan) or patterned fabrics, such as process control monitors, can also be used to evaluate the deposition of particulates on surfaces (May and Spanos, 2006).

Application to this study:

- Metal surfaces of Mars 2020: Partially effective; not directly applicable to tubes.
- On Mars-sourced rock and soil samples: Unknown.

Summary. Well-known methods exist for detecting Earth-sourced contaminant particles on smooth surfaces, and also for distinguishing organic from inorganic particles. However, we note that few if any of these methods are suitable to direct measurements on confined surfaces such as sample tubes due to access limitations and the possibility for introduction of contaminants to flight tubes, although some methods could potentially be applied indirectly via analysis of rinses of flight hardware or surrogates (*i.e.*, witness coupons). The OCP does not know of any fully effective methods for quantifying Earth-sourced organic particulate contaminants in rock and soil samples.

4.4.3. Limits on organic particulates. The OCP agreed that organic particulates should be identified and minimized on the sample contact surfaces of Mars 2020; however, the

Panel could not agree on what constituted a reasonable upper limit on the numbers of these particulates. In terms of protecting the science, much work has been done on meteorite samples contaminated with significant numbers of organic particulates and terrestrial microorganisms. Yet the spatial distribution and total numbers of particles are important when interpreting samples; contaminant particles transferred from spacecraft surfaces to cached samples could remain almost entirely on surfaces (albeit including cracks, etc.), providing a potentially robust way to distinguish *possible* contamination from indigenous particles. In addition, particles are less likely than single molecules to migrate into the interior of samples, leaving them more pristine. As such, having all of the contaminating TOC in the form of particulates would actually benefit our ability to recognize such contamination, although such particulates would not prove beneficial for extraction-based chemical analyses. On balance, the OCP felt there was no strong scientific reason to propose specific limits on the numbers of organic particles, beyond the expectation that the strongest efforts to minimize particulate contamination within sample containers. As a guide, these could include the most stringent industry standards, such as those used by the semiconductor manufacturing industry (May and Spanos, 2006).

Particulate contamination, by its nature, is quantized. Each particle would constitute of a substantial dose of molecular contamination. As discussed above in Section 4.1.5, it is likely that the requirement for the number of contaminating terrestrial microbes would need to be expressed in statistical terms. For example, counting a statistically representative number of particles in multiple surrogate containers or large-area witness plates might be needed in order to confidently predict the level in flight hardware. Such considerations place practical lower limits on the levels of particulate contamination that can be required. For example, we can conceive of limits on contamination that amount to <1 particle per unit area (or per sample tube, etc.) on average, but the best we can do is say there are zero or one particulate in any particular sample. To ensure statistical confidence, if we examine 1 m^2 of spacecraft surfaces, the lowest level of particulate contamination we could (confidently) detect would be $10/\text{m}^2$. If we sampled a surface of only 300 cm^2 (0.03 m^2), our limit would be $300/\text{m}^2$; at 30 cm^2 , it would be $3000/\text{m}^2$. Thus reducing sample-contacting surface areas has the unintended consequence of making it harder to detect very small numbers of particles. Given these statistical limits, we would not be able to verify that sampling hardware with 30 cm^2 of contacting surface area had <200 organic particles/ m^2 on average, the best we could do is $<3000/\text{m}^2$. This can be partially compensated for by combining data for multiple sample tubes, although this has practical limits. Fifteen of the above sample tubes would have to be pooled to reach the <200 particles/ m^2 detection limit; 150,000 would have to be pooled to reach a limit of <0.02 particles/ m^2 . Similarly, if we wished to establish the probability of contamination by less than 1 particle per tube at 1:1000, we would have to examine at least 1000 tubes and show that no more than 1 of them contained 1 particle.

A final consideration is that of particle size. The lower detection limit with electron microscopy is approximately 5 nm in diameter; however, detection of all organic particles

this size and above on large surfaces would be immensely burdensome. Therefore, we recommend a particulate size limit for observable detection of 200 nm based on the theoretical limit for independently living microbial life on Earth (200 nm), and the size of the smallest microbial cells observed in nature (approximately 300 nm) (Velimirov, 2001; Morris *et al.*, 2002; Miteva and Brenchley, 2005).

Finding #19: Due to statistical uncertainty in measurement and the potential to introduce further contamination through measurement, it is difficult to place a lower bounding limit on the allowable numbers of organic particulates.

4.4.4. Conclusions and recommendations for particulates. From the perspective of scientific objectives, organic particulate contamination would have both positive and negative impacts; particles on surfaces masquerading as life are of course confusing, and will degrade surface-based investigations, but are less likely to migrate into samples than are molecular contaminants. Given the difficulty in implementing a viable statistical assessment of contamination and the relative impacts of particulate organic matter on sample analysis, the OCP was unable to reach a recommendation on quantitative limits specific to organic particulate contamination on returned geological samples. Cleaner is clearly better, but the concept of “minimal level necessary” is not clearly meaningful in this context. It is expected that the very highest industry standards for limiting such contamination on sample-contact spacecraft surfaces would be used, but it is quite unclear how this would translate to sample contamination levels. The OCP noted that detailed characterization of the contaminating particles (type, abundance, etc.) will greatly help to reduce ambiguity in the future analysis of cell/spore-like structures in martian samples.

The OCP recommends that organic contaminant particles should be limited to levels as low as is reasonably achievable, and that particles must be included in the accounting for total organic contamination loading on sampling surfaces. Particulate cleanliness levels for semiconductor processes are on the order of $<0.1 \text{ ng}/\text{cm}^2$ (Tajima, 1993), and this may be reasonable for the sample-contact surfaces of Mars 2020. Particles 200 nm and larger should be characterized for chemical composition using a variety of methods such as those already identified for Tier-I contaminants and augmented by biospecific assays such as amino, nucleic acid assays, and culture-based methods where applicable.

Finding #20: If the contaminants are well characterized, then observed organic particulates could be rapidly differentiated as having a terrestrial origin. Thus, as above, we strongly recommend the extensive use of witness plates and surrogate hardware, along with extensive archiving to allow characterization of remnant particle contamination on sampling surfaces.

4.5. Implementation

4.5.1. Strategy for implementing contaminant requirements. The Panel proposes the following strategy for

implementing and verifying required organic contaminant limits. First, a robust analytical program should be set in place to characterize as many individual organic contaminants as possible at concentrations below 1 ng/g (total sample loading equivalent). This program would form the backbone of the contamination characterization that would be so essential to scientists studying the returned samples. The program should, at a minimum, be able to detect all Tier-I compounds at these levels, and the project should actively monitor the abundance of these compounds. We do not wish to identify analytical methods that *must* be used by the project, but suggest that mass spectrometric approaches [GC-MS, liquid chromatography–mass spectrometry (LC-MS), DART/MS, etc.] would be especially valuable here.

Finding #21: Methods used for assessing hardware surface contamination should be demonstrated to have a known, reproducible efficiency of collection of the target Tier-I compounds.

The Tier-II list of contaminants contains too many compounds to explicitly test all of them even once, much less many times. Our strategy here is that any Tier-II compound that is observed at >10 ng/g (equivalent sample concentration) as part of the rigorous contamination characterization program must then be reduced to <10 ng/g. However, explicit validation by measurement that every Tier-II compound is <10 ng/g should not—and in fact cannot—be required.

Finally, TOC on sampling surfaces should be measured by whatever method(s) the project feels are most appropriate, so long as they can assure the limit is met. Those that provide more detailed information about the molecular makeup of TOC are strongly preferred given the goal of contamination characterization. Although different analytical techniques can include different classes of material in the resulting TOC value (see discussion in Section 4.3), these differences are expected to be relatively minor and not worth explicitly stipulating.

Finding #22: The overall organic contamination control strategy should involve monitoring for Tier-I compounds, monitoring of TOC, and broadband screening for Tier-II compounds above 10 ppb.

5. Strategies for Recognizing and Characterizing Organic Contamination

5.1. Introduction

The OCP recognizes four broad strategies for recognizing and distinguishing organic contamination. Some of these strategies are also beneficial in the characterization of contamination:

1. Use of witness plates
2. Effective use of blanks or blank standards
3. Archival of organic and trace biological materials sent to Mars, to be used for reference in interpreting analytical data collected from the samples
4. Spatially resolved measurements on returned samples

5.2. Witness plates

Witness plates would be critically important to the integrity of the science if samples are returned. Witness plates are objects, such as metal or ceramic plates, that are positioned in a way that allows them to collect the same chemical contamination or debris as an object of interest. When exposed sequentially during the course of a mission, witness plates can establish a record of the history of contamination events. The witness plates can be analyzed to identify the type and quantity of contamination at various times and three-dimensional positions. In the case of the MSR mission, they could be used to provide information about both the flight and the ground environment, including operations on the ground during ATLO of the Mars 2020 rover, the flight environment, the recovery environment at the Earth landing site, the containment environment, and curation. This information would be a key input into distinguishing native compounds from introduced contaminants. Experience with the managing of other sample collections suggests that allocation requests for its witness plates are as common as requests for the samples themselves (for example, as of this writing there have been 600 allocations of Genesis solar wind samples and 300 allocations of the accompanying flight-like reference collectors).

Since samples and sampling hardware have different physical properties with respect to different contaminants, it is typically best to employ multiple (at least two) different types of witness plate material (Sandford *et al.*, 2010). This allows the witness plates to account for different adsorption and absorption properties among contaminants. It may be desirable to place one or more witness plates inside the sample tubes. In addition, some effort should be put into understanding witness plate materials and sizes, in order to optimize eventual analysis. Time-of-flight (TOF)–SIMS (static SIMS with “*in situ*” virtually non-destructive surface analysis of both organic compounds and inorganics) could be very useful for sample analysis (however, there are questions given the possible low organic content in the samples). However, it would be simply invaluable for *in situ* contamination characterization on hardware, witness plates, cache tubes, and other pieces of hardware.

While it is desirable for the witness plates to maximize surface area and mimic the chemistry of the sample, it is more important that the witness plates (1) do not endanger the mission, (2) do not contaminate the sample, (3) are amenable to a variety of analyses, and (4) can be divided between different labs without further contaminating them. For example, while zeolite is more martian-like than sapphire windows and is easier to divide, it may be harder to contain, and particles could get into the sample, or even endanger spacecraft mechanisms. A larger number of smaller witness plates may be preferable in the sense that not only can a larger number of witness plates be used with multiple instruments, they can provide a more statistically robust sampling (or a combination of both factors). Experience with previous missions (e.g., Sandford *et al.*, 2010) shows that witness plates cannot be easily divided after the mission without risking further contamination. Witness plates should be deployed with a geometry that provides a molecular “view” of the sample, or hardware they are representing, and thus, they should be located as close to the actual sample, or hardware as possible. Also, consideration should be given to

the possibility that cache hardware components might suffice as supplemental witness plates. Other important considerations include dimensions of the plates, mounting, transport to analytical instrumentation, and the development of procedural controls that ensure confidence in the analysis. These multiple trade-offs should be examined by the project team.

Finding #23: It is critically important that Mars 2020 have a logically designed, and systematically implemented, witness plate strategy. The witness plates collected during the Mars 2020 build and archived for future reference would be essential to possible future returned sample science.

The flux of contamination onto the Mars 2020 spacecraft and rover would not be constant, and accrued contamination may change through exposure to different environments. Also, not all contaminants are retained by the samples equally over time due to chemical variation and volatility. Thus, the more temporal/spatial information gleaned from control samples, the more precisely that information can be applied to understanding the samples. This temporal variability in contamination leads to the need for phased witness plates, and the sequence of witness plate employment can be roughly divided by mission phase. The detailed employment strategy of witness plates depends on the number of witness plates required to record the various phases of the mission, how many time periods should be sampled during the mission, the witness plate geometry and composition, and, ultimately, how many witness plates would be needed for post-flight analysis. We recommend that a witness plate campaign should be explicitly designed and implemented as part of contamination control for the mission. For Mars

2020, key time periods for sequential witness plate employment include (see Fig. 14):

- Assembly and test
- Launch, cruise, Mars EDL (entry, descent, and landing)
- Mars surface operations
- Mars extended storage
- Earth recovery operations
- Preliminary sample examination
- Long-term curation

Finding #24: In order to track the introduction of contaminants, Mars 2020 (and all successor missions and ground activities needed to present samples to Earth-based analysts) would need a carefully designed and systematically implemented witness plate program.

Beyond the considerations for the Mars 2020 sampling rover, witness plates should be used to record the contamination environment of individual samples in the SRF, and those witness plates should be periodically analyzed to maintain a contamination knowledge record for individual samples and sample subsets. One key item with respect to witness plates is the differentiation between witness samples collected (during build and up to launch) for future analysis and those collected that require analysis to validate cleanliness levels immediately so any mitigation steps can be performed. Witness plates integrated with the assembly of the sample retrieval and storage system would be the most useful. A subset of these plates can be pulled off and archived prior to launch and analyzed when the samples are returned and compared with the plates that made the trip. The idea of trying to put some of these archived plates through the same environmental changes the Mars plates are

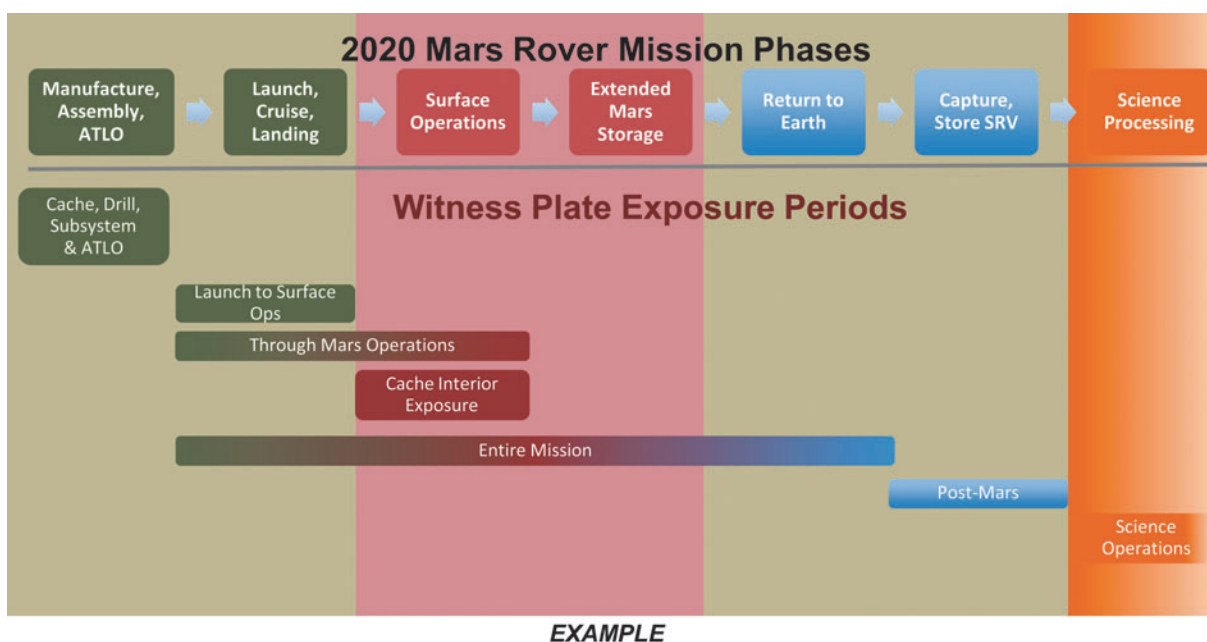


FIG. 14. An example of a witness plate deployment plan to provide information on the nature and timing of contamination events/processes. Note that for MSR this plan would need to be established early, since some of the witness plates that would be valuable later need to be deployed early, and make the round trip to Mars. SRV, sample recovery vehicle.

experiencing would give a good view of the changes various molecules go through during the trip.

An example of the structure of a witness plate plan is shown in Fig. 14. A key point is that this plan needs to be established early in the MSR mission, and then implemented systematically throughout—some of the exposure periods begin in the mission development phase. Representative witness plates should be archived for eventual analysis in parallel with returned martian samples. However, it would also be useful to analyze the assembly and test witness plates on short time scales to mitigate contamination events, as part of ATLO contamination control/knowledge. If direct sampling of contamination on flight system surfaces is prohibited owing to concern about recontamination or geometry, then witness plates of identical material (*e.g.*, 6061 aluminum) can be processed in parallel with the same cleaning and testing procedures to serve as a contamination control proxy.

5.3. Blanks and blank standards

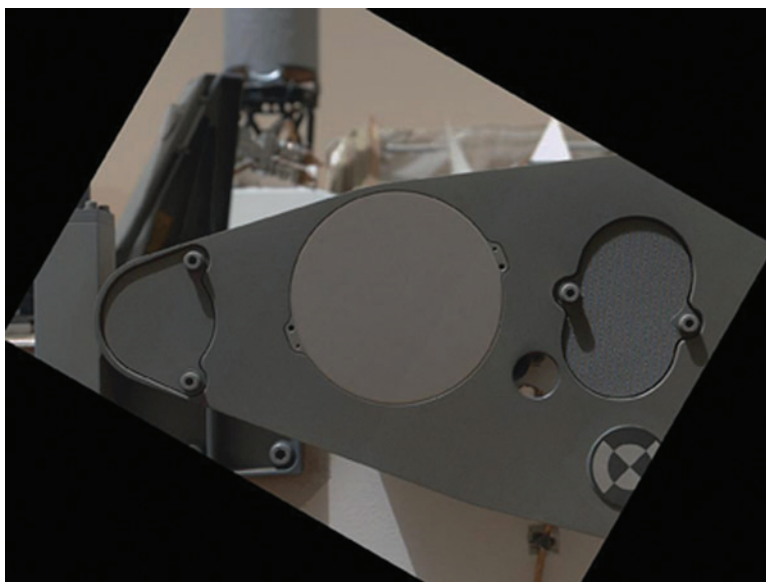
In contrast to witness plates, which provide an accumulation of contaminant particles and films during a defined period of time, a blank serves as a measurement of non-sample-related inputs to an instrument signal. *Blank standards may turn out to be the most sought-after material collected on Mars.* Without them, measurements of organic compounds in all other returned samples would be unconstrained and therefore suspect. In the case of the possible returned martian samples, a blank can be envisioned in one of two ways: (1) A system (collection, analysis, other) is operated as it would if a sample were present, except that no sample is inserted. This would be a crucial step for the instruments used to analyze the possible returned martian samples. For the sampling system of the Mars 2020 rover, this could mean that a sample tube is opened and then closed without inserting a sample. (2) A synthetic sample known to have zero concentration of the analyte of interest is inserted. Any instrument response to the analysis of such a sample can be interpreted as contamination.

As an example, Phoenix flew an organic-free ceramic blank that was to be used to characterize the cleanliness of the sampling system by using the TEGA to detect organic molecules (Ming *et al.*, 2008). MSL was prepared to use this strategy to control its sampling and analysis operations by means of what is referred to as the organic check material (OCM) (Conrad *et al.*, 2012), although as a practical matter, the OCM has not been sampled on Mars as of this writing (Fig. 15). The OCM is a block of ceramic (a non-Mars material) that was fired at high temperature to drive off all organic molecules. (The OCM was then doped with a single non-Mars organic molecule, so that it can serve both as a positive and a negative control standard.)

As shown in Fig. 16, there are several potential strategies for inserting blank standards into the sequence of unknown martian samples. The decisions regarding how many, and when blank standards should be added, are left to the Mars 2020 Science Team. Regardless of the actual strategy, it is important that the blank standard be collected periodically as the sample (*e.g.*, the geological unit examined) and/or the sampler (*e.g.*, the drill bit) changes (Fig. 16). It is desirable that sampled cores of the negative control standard bracket (in time) the collection of actual samples, with the allowable caveat that a sample collection system failure could interrupt collection of the final negative control. The key consideration is that in order for the potential detection of organic molecule that may imply past life on Mars, to have maximum credibility, such samples should be bracketed by standards.

Past studies have assumed that in a 31-slot cache, at least three slots would be reserved for blanks (see, for example, McLennan *et al.*, 2012; Mustard *et al.*, 2013). However, as shown in Fig. 16, it is not hard to envision scenarios in which this number appears to be too low, and it would seem more prudent to have the capability to be able to collect at least five blank standards, and possibly six. How this capability is actually used during the mission would be up to the Mars 2020 Science Team, and would presumably depend on what is encountered by the mission during its surface operations phase.

FIG. 15. NASA's Mars rover Curiosity carries five cylindrical blocks of OCM for use in a control experiment if the rover's SAM laboratory detects any organic compounds in samples of Martian soil or powdered rock. The blocks are carried on the front of the rover, within reach of the sample-collecting drill on the rover's arm, and are sealed under foil until needed. This image centered on the foil that covers one of the bricks was taken by the rover's Mars Hand Lens Imager during Sol 34 of Curiosity's work on Mars (September 9, 2012).



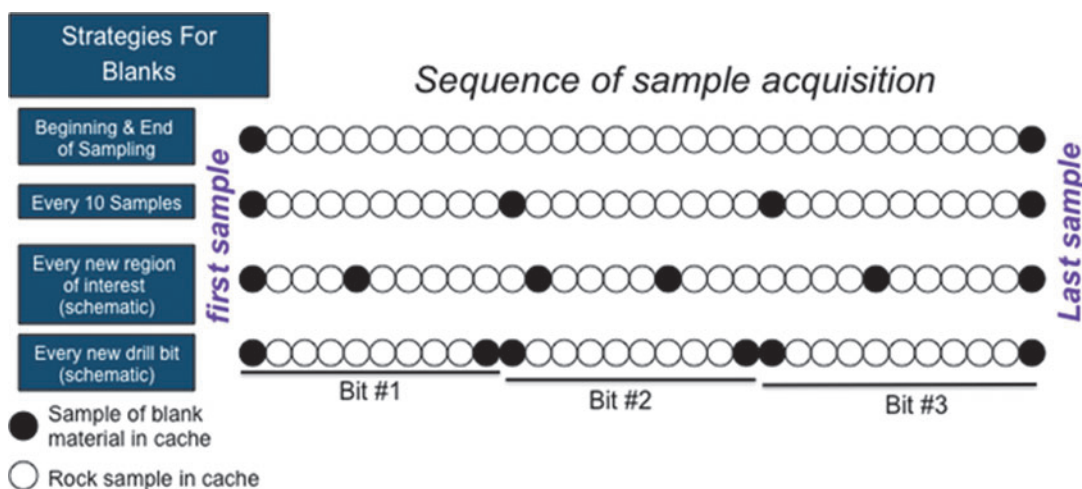


FIG. 16. In order to bracket all unknown martian samples with blank standards, the first sample taken should probably be a blank standard. The use of blank standards in the rest of the sample series depends on the decision of the Mars 2020 Science Team. Some suggestions are offered here.

Finding #25: The return of *in situ*-drilled procedural blanks is an essential part of the science of this mission. Proof of detection of Mars-sourced organic molecules in returned samples would not be convincing without them. OCP proposes that Mars 2020 have the capability to return six such blanks (although decision-making on how to use this capability is deferred to the operations team).

Note that *positive control standards* are also typically a critically important part of the sample analysis process. However, their value lies in validating the reality or quan-

tification of a positive detection, not in interpreting contamination, so they are not discussed further here (positive controls are typically synthetic materials that contain a known concentration of an analyte of interest).

5.4. Archive of organic and trace biological materials

The storage of archived materials is of great importance, and some careful thought needs to go into that aspect (since we need to be able to go back to these materials after the cache is returned and be reasonably certain that these materials and the contamination level recorded in them has not

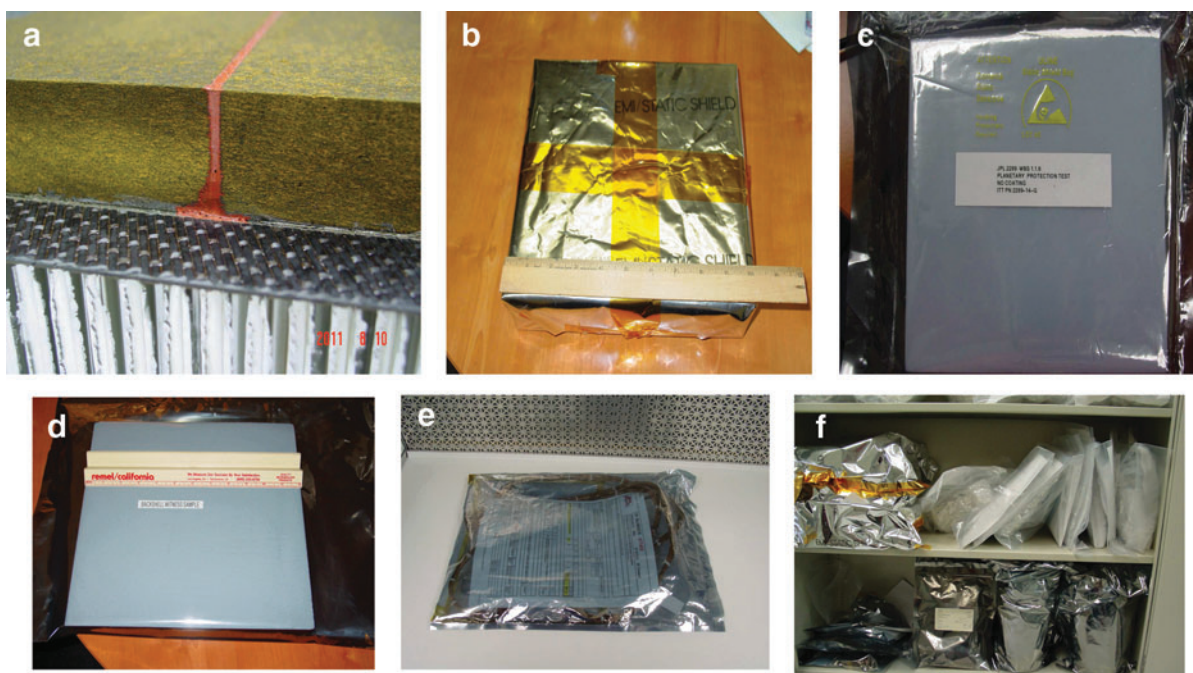


FIG. 17. Examples of MSL material samples, many of which are organic-bearing, stored in the JPL Planetary Protection Archive: (a) heat shield, (b) heat shield in ESD packaging, (c) heat shield panel, (d) backshell, and (e) harness. (f) The samples are stored in a cabinet under well-controlled temperature conditions. Source: Melissa Jones, JPL Biotechnology and Planetary Protection Group.

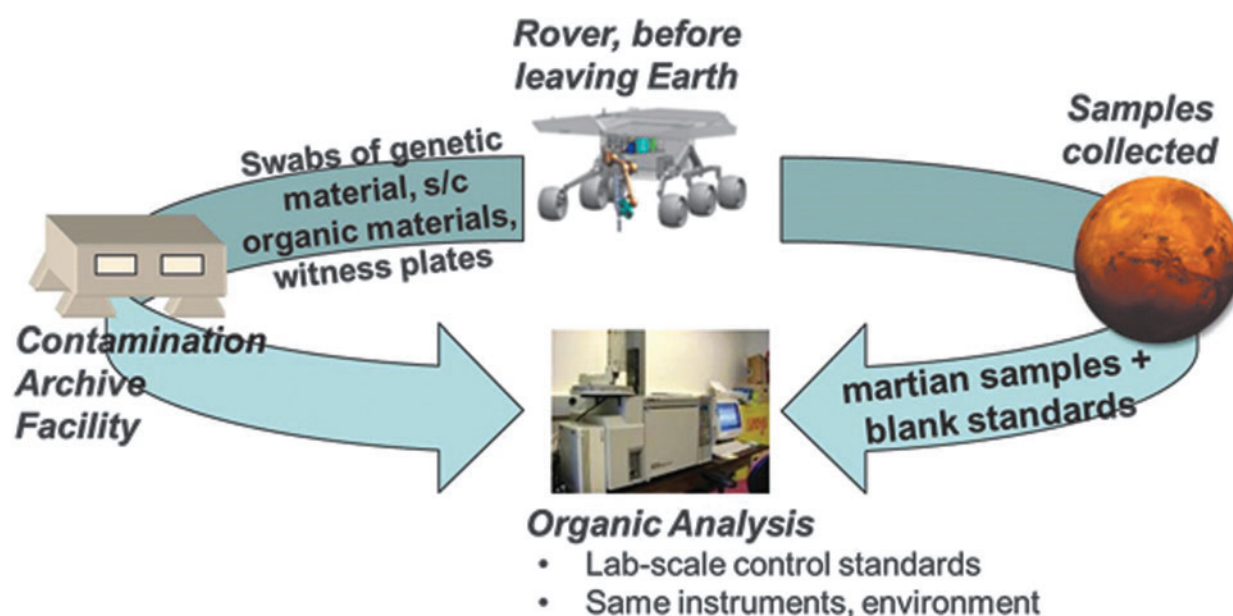


FIG. 18. Samples of organic matter on outbound spacecraft, including swab samples of trace microbial contamination, organic-bearing materials used in the spacecraft build, and witness plates, should be stored for future reference. Some portion of these samples presumably would need to be analyzed shortly after collection, but the main value would be analysis with the potential future martian samples and blank standards. (Modified after Mustard *et al.*, 2013).

changed significantly over the decade or more between launch of Mars 2020 and the return of the cache). Materials storage would support legacy analyses (Figs. 17 and 18). To support analyses, storage contamination effects must be clearly understood.

Appropriate witness plates, blanks, and flight-like reference materials are important and require adequate documentation and preservation. To correctly interpret round-trip contamination in returned samples, a facility and systematic approach for storing several kinds of materials and samples associated with the Mars 2020 rover would need to be established. All of the samples described below need to be preserved, monitored, and made available to analysts in a suitably clean, equipped, and staffed curation facility. In the case of organic analytes, particular attention needs to be given to the conditions of storage in the archive, requiring a well-documented systematic approach. Stored genomic samples would allow for capability to compare against returned samples to avoid false positives. The OCP is aware of two relevant capabilities already existing within the NASA system: (1) the facilities managed by the Astromaterials Acquisition and Curation Office at Johnson Space Center, and (2) the Planetary Protection Archive, managed by JPL Biotechnology and Planetary Protection Group. The latter archive currently houses approximately 200 organic material samples, approximately 100 flight parts/components (see, *e.g.*, Fig. 17), approximately 200 nucleic acid samples from MSL (Venkateswaran *et al.*, 2012; La Duc *et al.*, 2014), and approximately 3500 microbial isolates (Schubert *et al.*, 2003; Schubert and Bernardini, 2013, 2014).

1. Trace genetic material on outbound spacecraft. Dating back to the Viking era, the history of Mars exploration

has collected thousands of samples of microbial contaminants from Mars-bound spacecraft prior to their launch. There are several existing culture collections housing isolates derived from these samples, including ESA's collection at DSMZ (Moissl-Eichinger *et al.*, 2012), JPL's Phoenix research collection at the U.S. Department of Agriculture, Agriculture Research Service (Venkateswaran *et al.*, 2014), and JPL/Mars Program Office's Mars-related collection archived at JPL, under study in collaboration with the University of Idaho. These samples have allowed for phylogenetic studies of the variety of taxa (including bacteria, archaea, and fungi) that have the potential to have been sent to Mars (Venkateswaran *et al.*, 2012). In the case of the Mars 2020 sample-collecting rover, a similar set of samples should be collected and archived to support potential evaluation should Mars samples eventually be returned.

2. Witness plates (Section 5.2 above). As discussed above, witness plates and the negative control standards need to be maintained as long as the martian samples are cataloged and distributed through the same procedures.
3. Organic materials used in the spacecraft build. It is essential that samples of all organic-bearing materials used in the course of building and processing Mars 2020 hardware be collected and archived. This includes polymers used in electronic components, cleaning solvents, polymers used in spacecraft manufacture such as Kapton, mold-releasing agents, machine oils, system lubricants, etc. It is impossible to build a spacecraft, rover, collection system, and return system purely out of carbon-free materials. Motors require lubrication, surfaces require coatings, joints require adhesives, and

even stainless steel alloys contain low levels of metal carbides. At the time these samples enter the archive, they should be analyzed for a range of compounds identified as of special interest to Mars 2020 (*i.e.*, the Tier-I compounds). Prompt analysis would guard against degradation of the archived compounds before the cache is returned, and still retain material for analysis should a contamination contingency arise.

4. Samples from the possible future Earth landing site. Samples of the soil and air to which the capsule is exposed at Earth landing should be collected and archived. Materials from the sample return capsule ablative shield (if used), which may have organic components, should be collected and stored. (Note that this is not an issue for Mars 2020.)

Finding #26: Samples of organic and biological materials associated with the process of building the Mars 2020 spacecraft should be collected and preserved in a contamination archive facility. These samples should be available for analysis during a potential future returned sample analysis phase.

Within the archive facility (and on the flight mission as well), attention to packaging is extremely important. Avoid plastic bags as primary containers for cleaned hardware and returned samples. Packaging of cleaned parts is often done by heat-sealing inside plastic bags. The plastic films liberate plasticizers just by their presence. Even more plasticizers are given off when heat sealing. Of particular concern to this

mission is outgassing of caprolactam by nylon bags, as there is some indication caprolactam reacts with water to form amino acids.

Finally, experience from prior missions (*e.g.*, Genesis) has shown that nearly every handling step (measurement technique or cleaning step) can add contamination—this needs to be planned for.

5.5. Spatially resolved measurements on returned samples

Spatial information is one of the most powerful means of determining whether organics are contaminants or indigenous to the sample (*e.g.*, Allwood *et al.*, 2009; Steele *et al.*, 2012, 2013). Spatial information is also critically important for determining the origin of indigenous organics [biotic, abiotic, etc. (*e.g.*, Schopf, 1993, 2006; Ueno *et al.*, 2001; Sugitani *et al.*, 2007, 2010; Allwood *et al.*, 2009, 2013; Steele *et al.*, 2012)]. For example, if organics were distributed across only the exterior surface of a core, the probability that they are contaminants would be high. Similarly, if the organics reside in cracks that are connected to the exterior environment, it is possible that the organics are contaminants. As an illustrative example, Figure 19 shows evidence of terrestrial microbes growing in recent cracks within the martian meteorite NWA 7034. An indigenous origin for organic carbon would be indicated if the carbon is embedded in the mineral matrix, in an environment protected from exposure to contamination sources (*e.g.*, see Agee *et al.*, 2013). Similarly, if organic carbon deposits are

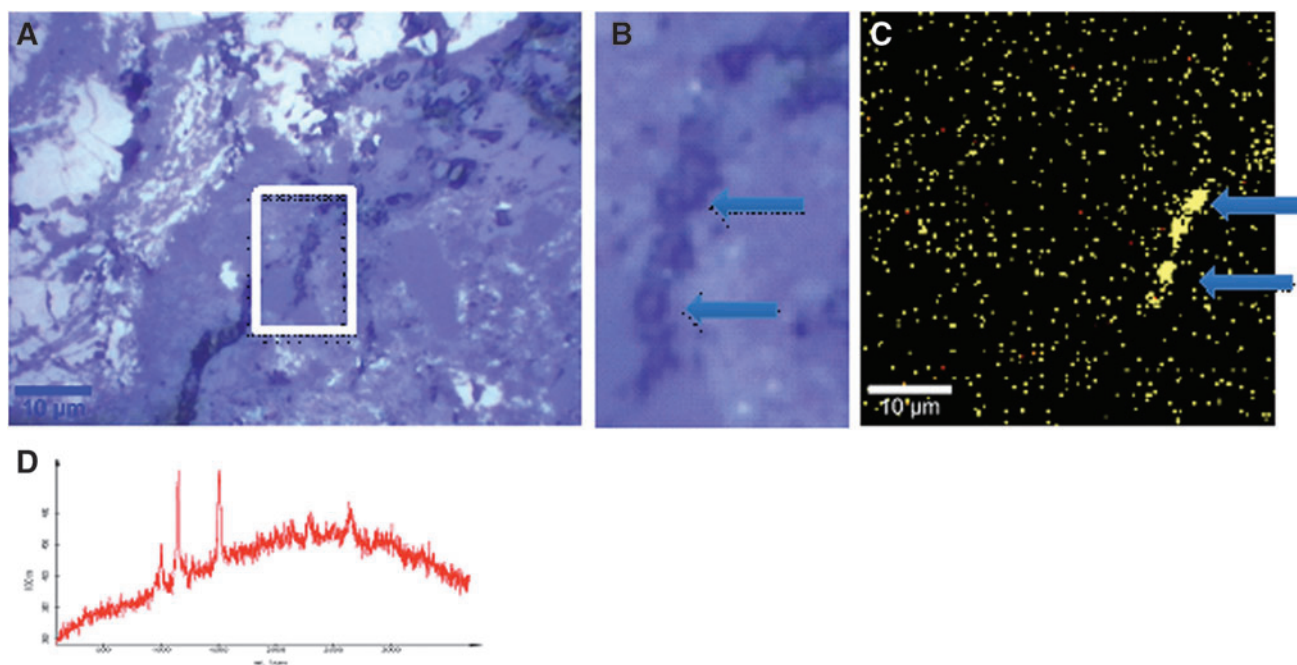


FIG. 19. The spatial arrangement of organisms within cracks and fissures of the martian meteorite NWA 7034 shows that has been exposed to terrestrial conditions for quite some time lead to the irrevocable conclusion that these cells (indicated by arrows) are terrestrial microbial contamination. (A) A reflected light micrograph. (B) An enlargement of the boxed region in (A). (C) A Raman peak image of β -carotene showing the location of microbial contaminants at the same scale as in (A). (D) Raman spectrum of β -carotene from a single spot from the map in (C). For additional information, see Steele *et al.* (2013).

cross-cut by geologic features in the sample, such as a vein or the broken edge of a clastic sedimentary grain, then those organic deposits are indigenous to the rock.

Finding #27: Maintaining the original physical structure of samples (*e.g.*, layering, gradients, grain boundaries, and cross-cutting relationships) as much as possible is extremely important to interpreting indigenous organic geochemistry.

As noted by Mustard *et al.* (2013), the location of organic molecules on/in the sample(s) and with respect to host mineral assemblages at the microscopic scale would provide crucial insight into the origin of these organic molecules (whether terrestrial contamination versus martian). Organic molecules located in the rock core interior may have a very different meaning than the same molecules found on the surface of the core. Surface removal or excavation (*e.g.*, ion beam milling/sputtering) combined with microanalytical capabilities (*e.g.*, nanoSIMS or TOF-SIMS) would be essential technologies for the analyses of returned samples. Mustard *et al.* (2013; Section 6.3.5.2) presented an important case history involving the Tissint martian meteorite that illustrates these points.

One aspect of this is that a priority for interpreting organic contamination in rock samples is to make them available to the analysts in good mechanical condition. If samples are pulverized, or even worse, the fragments move relative to each other, a significant amount of interpretive power would have been lost. In analogous terrestrial studies, it is common for the spatial distribution and morphology to play a significant role in the interpretation of the origin of organics (Allwood *et al.*, 2013). Therefore, maintaining the original structure of martian samples to the extent possible is crucial to enabling confident interpretation of the origin of any detected organics. Some important potential strategies to accomplish this goal could include minimizing fracturing of the sample during collection, restraining the cores within the sample tube to minimize vibration/shock damage, and marking cores in order to record the original geometry of pieces relative to the rest of the core. These issues are discussed further by Beaty *et al.* (2014) and Liu *et al.* (2014a,b).

6. Discussion and Proposals for Future Work

6.1. The case for cleaner

Sample return missions have a very special place in humans' exploration of the universe, in that they provide "ground truth" for all other types of investigations—computational, laboratory, and flight missions. As seen repeatedly in previous sample return missions, they often prompt fundamental shifts in our understanding. Apollo lunar samples revolutionized our understanding of the evolution and current state of the Moon (*e.g.*, Taylor, 1975). The Stardust mission provided the first physical evidence of pan-Solar System radial mixing in its formative stages (Wooden *et al.*, 2007). The Genesis mission has delivered the first direct measurement of the oxygen isotopic composition of the Sun, among other measurements (McKeegan *et al.*, 2011). All of these findings have proven invaluable in addressing current hypotheses about our Solar System.

Sample return missions provide uniquely valuable information that cannot be obtained in other ways. Fundamentally, sample return missions provide three important things: (1) the mission science team gets to select their samples, (2) sample context is well known on a range of scales, from knowing the originating body all the way to understanding the specific sampling site(s), and (3) the mission can carefully control and document the contamination history of the sample. Meteorites also provide samples, but they are biased towards tough materials that survive impact-generated transfer, arrive on Earth without geologic context, and become contaminated with terrestrial materials upon impact. So while they are valuable, from an organic standpoint, they are not a replacement for sample return.

Although sample return missions are very scientifically valuable, they are also inherently very difficult, and MSR will be especially so. There is thus a strong case to be made that the MSR program is not merely an opportunity, but an extraordinary opportunity. Although we often talk about scientific success or failure, the reality is considerably more complex. The more scientific measurements we are able to make (*i.e.*, measuring signals that clearly exceed contamination backgrounds), the more we will learn. Indeed, it is conceivable that we could learn a great deal more from these samples than the minimum required to declare scientific "success." There is thus a strong case to be made that we should not set contamination requirements at the highest possible level that still allows scientific success, but rather should strive to reach levels that are commensurate with the extraordinary nature of these samples.

As discussed earlier, results of previous missions (Viking 1 and 2, Phoenix) were originally interpreted to show that native soils contain vanishingly small amounts of carbon (Biemann *et al.*, 1976; Boynton *et al.*, 2009; Ming *et al.*, 2009). The confirmed presence of perchlorate, however, complicates all past interpretations because perchlorate can act as an oxygen source for combustion and a chlorine source to chlorinate molecules. Furthermore, the results of experiments on Mars analog soils cast further doubt on the earlier interpretations of miniscule organic carbon contents (Navarro-González *et al.*, 2006, 2010, 2011). MSL has now analyzed several different, martian sedimentary materials. The initial results for the aeolian drift Rocknest soil and the John Klein and Cumberland mudstone samples from Yellowknife Bay suggested that their carbon contents were either too low, or the data were rendered ambiguous by the chlorination and oxidation chemistry acting on the leaked derivatization reagents (Leshin *et al.*, 2013; Ming *et al.*, 2014). Subsequent data for the evolved gas and combustion experiments conducted by SAM, however, allow for the possibility that some organics detected in analyses of the Cumberland mudstone are martian (*e.g.*, Glavin *et al.*, 2013; Freissinet *et al.*, unpublished data). MSL is an ongoing mission, and as new results from the SAM instrument suite develop, our understanding of Mars organics is likely to change. We must also keep in mind that Gale Crater is not likely representative of the natural variability of Mars sedimentary environments. It seems clear, therefore, that we should remain open minded about both the upper and lower bounds of organic carbon contents of Mars sediments.

Based on this and other evidence, the current OCP and previous organic contamination-related panels have recommended upper limits in the single nanogram/gram range for especially important compounds, and upper limits overall in the 10–40 ng/g range. It seems likely that such levels will allow many of the most abundant organic compounds to be measured confidently. On the other hand, it is also likely that such levels will be problematic for at least some (and possibly many) trace organic compounds. Could we do more? Fortunately, the state of the art for contamination control has improved considerably over recent years, led in part by the semiconductor industry's need for particulate- and NVR-reduced materials and processes. Commercially available sample handling hardware regularly achieves ISO 1/2 particle cleanliness levels (as per ISO 14644-1). In terms of NVR cleanliness, lunar sampling boxes used in the Apollo program achieved 10–100 ng/cm² NVR cleanliness over 40 years ago (Calaway *et al.*, 2014). More modern publications state detection limits of various organic compounds to ppt levels, so analytical techniques are currently available to meet verification needs (*e.g.*, Fujimoto *et al.*, 2007). Also, NASA has the benefit of recent contamination control experience in contamination-sensitive missions such as Genesis, the James Webb Space Telescope, and the MOMA instrument for the ESA ExoMars rover. It therefore seems likely that achieving much cleaner levels of contamination is possible at reasonable expense. Although they may not be absolutely necessary, they could mean the difference between successful versus extraordinary scientific results.

Finding #28: Since we don't know the concentration of the organic molecules of interest in the martian samples that might be returned, there is an unquantifiable scientific risk relating to detectability above background. The cleaner (or dirtier) the samples are, the more (or less) compounds we would be able to measure, and the more (or less) confident we would be in interpreting their origin. Scientific return versus sample cleanliness is a continuous function that is hard to cast in the terminology of required/not required, or success/failure.

6.2. Summary and conclusions

We do not know what organics we would find in martian samples that are returned to Earth. This fact alone makes predicting the required cleanliness of returned samples highly ambiguous. Although we have attempted to constrain the problem to within an order of magnitude, we emphasize that much uncertainty remains. Given the high sensitivity of modern analytical techniques (detection limits of <1 pg), setting contamination limits that are low enough to remove any risk of interfering with any scientific measurements is not achievable. Thus choosing a discrete limit within the bounds we provide would implicitly require accepting some level of scientific risk (*i.e.*, that analyte concentrations in the returned samples are lower than we have anticipated). The panel members varied substantially in their opinions about the level of scientific risk that is appropriate for this mission, and our recommendations fall somewhere in the middle of this range. Several panelists in particular felt that the limits

should be substantially more conservative, to ensure a higher likelihood of ultimate scientific success, albeit at a (presumably) higher cost. Resolving such questions should involve a full consideration of costs and benefits associated with different levels of cleanliness, but our group was not able to evaluate the cost side of that equation.

The first logical step in setting limits is to determine what kinds of organics we care most about, and here our recommendation is that measurements of the individual organic molecules that would be the most scientifically valuable must be made. These are the measurements that should be most carefully protected from interference by Earth-sourced organic contaminants. A limit on TOC can play a supporting role as a blanket insurance policy against all possible contaminants, but should not be viewed as an end unto itself. Rather, any limit on TOC should be tightly linked to the perceived need to limit individual contaminants. We also agreed that virtually all organic compounds are of potential concern, either because we might potentially find them on Mars, or because they might plausibly interfere with measurements of martian organics. At the same time we recognize that certain molecules (mainly those known to be associated with terrestrial life, and those that have been detected on Mars) are of greater concern, and so recommend a two-tiered strategy for controlling individual contaminants: Tier I (see Table 4) contains compounds of greater concern, and so should have lower limits. Tier II includes everything else.

Our goal is to limit individual contaminants to levels that are below those we hope or expect to measure, but unfortunately we do not know which organic compounds would be present in those samples, nor at what concentrations. Previous groups have attempted to constrain the problem using estimates of meteoritic input fluxes, or by comparison with organic-poor terrestrial rocks. This Panel chose to rely more strongly on recently published measurements of martian meteorites and from the MSL rover; although few and far between, these measurements are at least demonstrably representative of martian materials. The Panel also considered the analytical techniques likely to be used on returned samples, and their sensitivities to various contaminants. However, because of the huge diversity of techniques, with detection limits spanning many orders of magnitude, this does not appear to provide useful constraints on allowable contamination levels. Our recommendations are consistent with protecting those measurements most likely to be used for initial characterization of samples (survey techniques), but would still be visible to other more targeted techniques.

The most valuable data for predicting expected concentrations, in our opinion, are the recent measurements of amino acids and TOC in martian meteorites, and the “tentative” detection of chlorobenzene by MSL. We infer that at least some, and perhaps most, martian rocks would contain important organic compounds at levels of a few to tens of nanograms per gram, and TOC at tens of micrograms per gram. If we assume a distribution of compound classes similar to that seen in the Murchison meteorite, then many other types of compounds should also be present at similar levels. These levels could be confidently measured against a background comprising <1 ng/g per compound. Given the small number of data, we point out that there is much uncertainty about whether these are typical values, or whether returned samples might have higher or lower concentrations.

Clearly having cleaner samples would provide the opportunity to measure more (lower abundance) compounds in more organic-poor rocks, thus maximizing scientific return. Nevertheless, adopting <1 ng/g per compound as a guideline should allow robust measurements in some rocks and so provide the ability to meet stated scientific goals.

A significant problem in implementing contamination limits lies in the vast number of potential contaminant compounds, which are far too numerous to quantify individually. One potential approach would be to limit TOC itself to below 1 ng/g. This is the most conservative approach, and thus the most scientifically desirable one. Although technologically feasible, it is difficult at the levels required and thus expensive. A second approach would be to explicitly monitor and limit Tier-I compounds at <1 ng/g, and TOC at <10 ng/g. This would ensure that all Tier-II compounds are present at <10 ng/g. The third and least conservative approach is to explicitly monitor Tier-I compounds, passively monitor Tier-II compounds at <10 ng/g, and then relax the TOC limit to 40 ng/g in order to limit the number of contaminants that can simultaneously be present.

There are several important strategies for recognizing terrestrial contamination in returned samples. First and foremost is a comprehensive plan of contamination monitoring and characterization during spacecraft construction. We would not achieve non-detectable levels of cleanliness, and it is essential to know—at the molecular level—what residual contaminants are being carried along. Second, witness plates can be used to monitor ongoing contamination during all stages of the project, and some of these would need to make the round trip to Mars. Experience dictates that these would be in high demand by scientists studying returned samples. Third, we deem the sampling (on Mars) and return of negative control standards (blanks) to be absolutely essential to building convincing evidence for the identity of martian organic molecules. We recommend planning to include multiple blanks on the rover. Fourth, an archive of all materials used to construct the spacecraft should be developed to provide future scientists with the ability to look for novel sources of contamination. Maintaining such an archive for decades, without further contamination, would be nontrivial. Fifth, a strategy that would undoubtedly be employed to assess the indigenous nature of any organics is their spatial distribution in the samples. For example, contaminants transferred from hardware are more likely to be present on sample surfaces. Preserving the original structure of samples during the caching and return operations would be of the utmost importance.

6.3. Topics for future work

During its deliberations, the OCP recognized several issues that would benefit from either further technology development, or further discussion by a subsequent group. Note that some of the items in the lists that follow are directly relevant to the proposed Mars 2020 rover, and others relate to other aspects of potential returned sample science. The lists that follow are not listed in priority order.

Of relevance to Mars 2020:

1. Further discussion is needed on the design of the so-called “blank standard” that could be sampled by the

Mars 2020 drilling system on Mars. MSL’s OCM is composed of a porous silica ceramic that is devoid of all organic molecules except that it is doped with fluorinated hydrocarbons (3-fluorophenanthrene and 1-fluoronaphthalene) in order to ensure that the lack of other detectable molecules is indeed due to their absence rather than a sample delivery failure. Thus, it serves as both a positive and a negative control standard. Many of the considerations that went into the design of the OCM would be identical for Mars 2020, but we do not yet know if all considerations will be identical.

2. More study is needed to determine the optimal solvent mixture for detecting all of the Tier-I compounds using swab samples. As part of this, the extraction efficiency of the chosen solvent mixture with respect to each of the compounds should be determined.
3. The transfer coefficients for organic molecules from sample contact surfaces to geological samples have been experimentally determined for abrasive transfer using granular samples (*e.g.*, Mahaffy *et al.*, 2004). However, there are insufficient data for the transfer of contaminants to solid core samples using a mechanical configuration relevant to Mars 2020. Transfer from the container walls to the sample by repeated thermal cycling (a condition the samples would experience while being stored on the surface of Mars) also has not been studied. OCP proposes a carefully designed set of experiments.
4. The possibility that trace Earth-sourced inorganic or organic compounds could alter or destroy martian molecules of interest was not evaluated by the OCP. If this becomes a significant concern, it would need to be evaluated by a successor group.
5. The OCP carried out its analysis based on molecular measurements that it anticipates would be made on returned samples. However, in order to set limits on concentrations of these same molecules on the outbound spacecraft, we assumed there is no degradation or modification of these molecules by the martian environment. If this is recognized in the future as a significant concern, it would need to be considered by a successor group.

Of significance to returned sample science, but not to Mars 2020:

6. In order to be able to make molecular measurements on samples as clean as those described in this report, NASA (and/or other interested space agencies) would need to invest in research and technology to develop and build the infrastructure that comprises the necessary analytic environment (including sample management, sample preparation systems, and instrumentation). To within OCP’s knowledge, no such labs currently exist on Earth.
7. OCP was asked to work with the assumption that the contamination of samples in the SRF would be small relative to the contamination they would receive during the flight mission(s). This needs to be systematically evaluated. It is far from clear to us what are the implementation implications for the Earth-based

sample environment of the limits described in this report (e.g., Tier-I and Tier-II molecules).

8. We need more research into ways of interrogating individual particles on the returned samples, that may be either organic or inorganic, and that might “look” like microbial cells. We have to remember that the samples would be collected in an environment (the drilling environment) that generates massive amounts of (Mars-sourced) particles, so the rock samples would definitely come back covered with lots of particles (although these particles may not constitute “contamination”). An essential question for the potential future Earth-based analysts would be whether any of these particles are “round-trippers” that originated from Earth. These questions are likely to come up on a particle-by-particle basis. Is there a way to assess this short of micron-by-micron mapping of the samples?
9. A comment made during the review process (note that because this is outside the scope of OCP’s charter, it is presented here without either endorsement or discouragement by OCP): Establishing a process for independent oversight for contamination control could be beneficial. Details matter with contamination control—an independent set of eyes as things are fabricated is important. In order to maximize the science return of the samples to be used for life detection, scientists using or familiar with the techniques that would be used for organic detections on returned samples should be in the loop of design, fabrication, and testing.
10. If samples are returned, we would have a critical need for careful planning to avoid inefficient and wasteful consumption of limited samples. For example, the choice of method for distinguishing dead organisms, inorganic carbon compounds, and viable microbes is an important detail.
11. At least some of the organic molecular measurements in the SRF would be time-critical, because the information would be relevant to interpreting whether the samples are hazardous or not. As such, these measurements would need to be done in containment. The technical issues associated with integrating a super-clean analytic environment into a containment environment may be quite challenging. Agreeing on a strategy for solving this may be one of the long-lead elements of the SRF design, so early attention is warranted.

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2014 Gordon Research Conference on organic geochemistry (where a poster was also displayed)—we thank those who interacted with members of the committee on this subject matter. The OCP acknowledges the intellectual contributions of Marc Fries during the first part of its deliberations. The OCP gratefully acknowledges assistance with the instruments and measurements tables from Sabrina Feldman (JPL) and Lauren DeFlores (JPL). The OCP also wishes to acknowledge the technical contributions made by Mark Anderson (JPL) and Ira Katz (JPL) in the areas of organic contamination measurement and redistribution analyses.

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A. Appendices and Supporting Files

Appendices 1, 2, and 5 are available online at http://mepag.jpl.nasa.gov/reports/OCF_2014_final_report_docx.pdf.

A.3. Appendix 3: Definitions of terms

Organic carbon—for the purposes of this report, any carbonaceous substance that is not inorganic. Typical

definitions include the presence of covalent C-C and/or C-H bonds, average oxidation state <4 , yielding CO_2 upon combustion, and others. All of these definitions comprise (different) subsets of the broader definition that we adopt here. Examples include formic acid, ethanol, glucose, hydrocarbons including methane, lipids, amino acids, purines, pyrimidines, urea, chlorofluorocarbons, Teflon, dimethylsilicone, etc. The term organic carbon does not imply formation by a biological process.

Inorganic carbon—the boundary between “organic” and “inorganic” carbon is ambiguous, and no single definition is broadly accepted. Here we use “inorganic” to refer primarily to materials comprised of oxygen and carbon. Examples include gaseous CO and CO_2 , dissolved CO_3^{2-} and HCO_3^- , and carbonate minerals such as calcite and dolomite. Many definitions of inorganic carbon also include metal and metalloid carbides, cyanides, and elemental carbon, although for clarity we refer here to such materials specifically by name rather than as inorganic carbon.

Elemental carbon—materials that contain only the element carbon, such as graphite, diamond, fullerenes, and graphene.

Macromolecular organic carbon—complex, high-molecular-weight, organic carbon compounds that are formed by polymerization or cross-linking of smaller subunits. Organic macromolecules include ordered biopolymers such as proteins, DNA, polysaccharides, and lignin; synthetic polymers including polyester, PTFE (Teflon), and silicone; and irregular geopolymers, such as humic acids, asphaltenes, and kerogen.

Organic particulates—macromolecular organic material that can be captured by sieving filters (for example, $>1\text{-}\mu\text{m}$ particulates).

Biologically relevant functional groups—atoms other than C or H in an organic molecule that impart functionality to the compound. Examples include alcohols, carboxylic acids, amines, amides, esters, and phosphate esters. Carbon-carbon double bonds are typically included in this definition.

Amino acid—organic carbon compounds that contain both an amine and carboxylic acid functional group. The linking of amino acids via a peptide bond $[(\text{C}=\text{O})-(\text{NH})]$ allows the formation of peptides and proteins in terrestrial biological systems. Terrestrial organisms use only 22 standard amino acids of specific chirality, although many more such compounds exist. Examples include alanine, cysteine, glycine, etc.

Carbohydrate—organic carbon compounds with the generic formula $(\text{CH}_2\text{O})_n$, containing multiple hydroxyl and carboxyl functions. Individual monomers (aka monosaccharides, sugars) can be polymerized via acetal and hemiacetal bonds to form polysaccharides (carbohydrate polymers). Examples include glucose, sucrose, cellulose, and starch.

Lipid—lipids, in comparison to “hydrocarbons,” are generally inferred to be of biologic origin. They commonly comprise long, hydrophobic hydrocarbon backbones with a polar end group and few functional groups. They can have linear chains (e.g., fatty acids, leaf waxes), branched chains (phytol, methyl-branched fatty acids), cyclic moieties (e.g., alkyl benzenes), or polycyclic moieties (e.g., sterols, lignin).

Hydrocarbon—formally, any molecule containing only the elements H and C. However, usage has expanded to

include any hydrophobic molecule originating in rocks or fossil fuels regardless of composition (e.g., “this rock contains $5\text{ }\mu\text{g/g}$ extractable hydrocarbons”). For this report, we adopt the latter meaning, and use it in conjunction with “lipids” to distinguish between biotic and abiotic sources.

Chirality—a characteristic stemming from the three-dimensional nature of organic carbon compounds. When a carbon atom is surrounded by four different moieties, it can exist as either of two non-superimposable mirror images (enantiomers). Enantiomers can rotate plane-polarized light in opposite directions and are so designated as “right-” or “left-handed” based on this property.

Homochirality—a collection of structurally similar molecules that are chiral in the same sense [*i.e.*, all left-handed (amino acids in terrestrial life) or all right-handed (sugars in terrestrial life)]. Homochirality is considered a characteristic of terrestrial biological systems.

Chain-length preference in lipids—the synthesis of lipids requires the addition of carbon atoms to a precursor to increase carbon-chain length. In biological systems, these carbons come from two-C donors (such as acetate) or five-C donors (isoprenoids), forming long-chain carbon skeletons with specific chain lengths. Compounds formed from acetate show strong preferences for even or odd numbers of carbon atoms (e.g., C_{12} , C_{14} , C_{16} , C_{18} , etc., in fatty acids, or C_{27} , C_{29} , C_{31} , C_{33} , etc., in hydrocarbons).

Pyrolysis products—organic compounds generated when a sample is heated, in the absence of oxygen, to the point of thermal decomposition.

Volatile and semi-volatile organic compounds—molecules with substantial vapor pressure either at room temperature (volatile) or at some elevated temperature (semivolatile). Molecules that thermally decompose before entering the gas phase are termed involatile. There is little agreement on precise temperature cutoffs between these categories; hence we adopt the practical definitions above.

Isotopes—atoms of the same element having a different number of neutrons, and hence mass. They are chemically identical and form the same compounds, phases, etc., but the mass difference causes them to react at subtly different rates. Radioactive versus stable isotopes (^{14}C vs. ^{13}C , ^3H vs. ^2H) are frequently distinguished, and the relative abundance of certain isotopes (in organic matter, primarily ^2H , ^{13}C , ^{15}N , ^{18}O , and ^{34}S) are frequently used to distinguish between materials of terrestrial versus extraterrestrial origin.

Isotopic fractionation—any chemical, physical, or biological process that alters the relative abundance of isotopes in a material. An example is the depletion of ^2H and ^{18}O in water vapor evaporating from a liquid. Many natural processes have characteristic isotopic fractionations (e.g., fixation of CO_2 in the photosynthesis). The loss of radioactive isotopes (e.g., ^{14}C or ^3H) due to decay is not typically regarded as fractionation as it occurs regardless of physical or chemical processes.

Contamination terminology

Organic contamination—any substance that significantly interferes with our ability to detect the presence of martian organic compounds or prevents our confidently determining that an organic compound is of martian and not terrestrial origin.

Constant contamination—background levels, such as in a blank, which are well characterized, constant and can be readily addressed in the evaluation of the compositional analysis. These are often mitigated or controlled by design and selection of materials and processes.

Random or variable contamination—spacecraft are huge systems requiring long periods of building. As a result, there is the potential for contamination to be introduced from entirely unpredicted events (“black swan” events). Such variable contamination can be identified, limited, or controlled by continuous monitoring of processes, systems, and witness plates.

Adventitious carbon—when surfaces are cleaned to a high level, the removal of surface oxidation layers, etc., results in the formation of a charged surface. Adventitious carbon comprises the charged carbon molecules within the atmosphere that are attracted to and bind to cleaned surfaces; therefore the chemistry of this carbon reflects the conditions of the environment in which it forms.

Contamination control—limiting the introduction of contaminants through processes and design.

Contamination knowledge—the use of witness plates, controls, and process monitoring to quantitatively and qualitatively characterize and understand the types of contamination such that interpretation of acquired data is possible and the science objectives can be met.

Contaminants of concern—the organic molecules identified by our scientific understanding of the environment, bioburden, and process design that provide the best indication of contamination that could interfere with the anticipated sample analyses and defined scientific objectives.

Surface contact transfer—the transfer of contaminants from a sampling surface to the sample. While the efficiency of this transfer is variable (depending on the types and nature of the contaminants and sample matrix), in a worst-case scenario it is assumed to be 100%.

Blank—a measurement designed to establish the amount of analyte due to sources other than the sample. Blanks can have many different contributing components, which may or may not be distinguished (*e.g.*, sample handling and storage blank, processing blank, reagent and solvent blank, instrument blank, etc.). Can also be referred to as a **negative control standard**.

Background—signals detected by the instrument that are due to sources other than the targeted analyte, for example, fluorescence or adsorption of sample matrix in optical techniques, contaminants present in the vacuum system of mass spectrometers, etc. The term is often, although not always, used to denote signals that interfere with or degrade measurement capabilities.

Witness plate—provides a background measurement alongside sample measurement to document where, when, and what contaminants are introduced during the mission. Witness plates generally comprise more than one type of material, each having different adherence properties (such as sapphire and silicone wafers), and can include clean plates, organic check material, or stored materials.

Pristine—in the context of sample collection, pristine can be considered as the level to which background contamination can be removed to within the cost and technical limitations of the time.

Noise floor—the lowest, reasonably achievable limit of contamination.

Analytical terminology

Analyte—the element, isotope, compound, substance, etc. of interest in an analysis.

Sample matrix—the sample material that surrounds and contains the analytes of interest (*e.g.*, sediment, rock, water, etc.). The sample matrix affects the manner in which sample is prepared and introduced into a measurement technique (*i.e.*, liquid vs. solid-phase extraction), as well as potentially affecting the analytical measurement itself.

Detection limit—is by convention defined as the quantity of a material yielding a detected signal at some specified level above the blank or noise in the measurement (signal/noise ratio). This may be regarded as the minimum level at which there is sufficient certainty in the measurement to state that the analyte is unambiguously detected, and as the maximum level to state that the analyte is not there. Different signal/noise ratios are adopted for different applications, but typically vary between 3 and 20.

Sensitivity—the amount of analyte required to provide a unit of measurable signal (*i.e.*, picomoles/mV). This term is often confounded with detection limit.

Resolution—the ability to separate or distinguish adjacent signals or compounds. The term has various meanings in different analytical techniques (*i.e.*, in chromatography refers to the ability to separate distinct molecular structures, whereas in spectroscopy refers to the ability to distinguish different wavelengths).

Quantitative analysis—an analysis carried out to measure the amount (or concentration) of analyte in a sample. This is typically achieved by comparing the instrument response from the sample to a calibration curve generated from authentic laboratory standards, although other approaches are possible. Note that the term does not imply that a measurement is free from error or uncertainty.

Qualitative analysis—an analysis carried out to determine the identity, structure, functionality, or other properties of the analyte. Because generating calibrating curves for quantitative analysis typically requires knowing what analytes are targeted, qualitative analysis typically precedes quantitative analysis in the study of unknown materials. Estimates of relative abundance from (typically uncalibrated) qualitative analysis are sometimes called “semi-quantitative,” although this term is ambiguous.

Analytical techniques

Chromatography—a family of techniques that relies on different rates of migration of analytes in a fluid phase travelling in a solid or liquid phase, for physically separating analytes in a mixture. The separation relies on differing physical and/or chemical properties of the analyte, such as vapor pressure, solubility, hydrophobicity, ionic strength, size, shape, etc. Techniques for organic separations are often distinguished based on the mobile phase used for the separation [*i.e.*, gas chromatography (analytes in a gas phase) vs. liquid chromatography (analytes in a liquid phase)].

Capillary electrophoresis—a family of analytical separation methods performed in a narrow bore (capillary) where

the analytes are separated by migration through an electrolyte solution under the influence of high electric fields.

Magnetic resonance—a family of techniques [generically nuclear magnetic resonance (“NMR”)] that detect the absorption and reemission of electromagnetic energy by atoms in a strong magnetic field, due to spin-flipping of nuclei. The technique is non-destructive and is widely used for structural elucidation of unknown organic compounds.

Mass spectrometry—a family of analytical techniques based upon the ionization of molecules, followed by manipulation, separation, and detection of those ions in magnetic and/or electrical fields. The technique typically yields the mass/charge ratio of each ion, which is useful in determining identity and structure. A variety of different ionization methods (*e.g.*, electron-impact, chemical ionization, photoionization, electrospray, matrix-assisted laser desorption/ionization, secondary-ion impact, etc.) and mass analyzer designs (sector-field, quadrupole, ion trap, TOF, Fourier transform-ion cyclotron resonance, etc.) can be combined. Hyphenated techniques with chromatography (*e.g.*, GC-MS and LC-MS) are very common. Techniques using multiple stages of ion manipulation (*i.e.*, MS-MS or MSⁿ) are sometimes used to increase specificity of analysis, or to help elucidate structure. Mass spectrometry is considered a “destructive” analytical technique.

Optical spectroscopy—a family of analytical techniques that work by observing the interaction of photons (light) with the sample. Techniques can include measuring light reflection or scattering, absorption, fluorescence (absorption and re-emission at a longer wavelength), and Raman scattering (scattering with a minor energy loss arising from stimulation of a vibrational mode). Observations at different wavelengths target different properties of molecules, with x-ray wavelengths targeting atomic (elemental) composition, UV and visible light targeting molecular electronic transitions, and IR wavelengths targeting molecular rotations and vibrations. Techniques can sometimes provide spatially resolved analysis, as in Raman microscopy. Optical techniques are typically non-destructive.

Mass spectroscopy—a mass/charge versus relative intensity plot used in chemical analysis. Typically, mass spectra are formed using a mass spectrometer when an organic carbon compound is ionized, decomposes according to the laws of chemistry. The fragments are separated according to their mass/charge, counted, and viewed as a relative abundance plot. Mass spectra, obtained under identical conditions can be a rapid, reliable, and sensitive means of identifying unambiguously identifying organic carbon compounds.

Total carbon/total organic carbon analysis—related techniques for the analysis of bulk materials that aim to determine total levels of (organic) carbon via combustion of analytes to CO₂, with quantitation of the evolved CO₂. Because the analysis is operationally defined (*i.e.*, anything that yields CO₂ at a given temperature), techniques that differ in temperature, time, pressure of O₂, etc., can include or exclude different materials. For example, graphite would be detected in a total carbon analysis at 1000°C but not at 500°C.

Laser desorption—the process by which incident laser radiation results in the separation of a molecule from a surface or matrix, allowing sampling of molecules with

fewer matrix effects. This process may result in ionization of the molecules.

Secondary ionization mass spectrometry (SIMS)—a family of techniques in which samples are sputtered and ionized by the impact of a beam of primary ions, typically followed by mass spectrometric analysis. They are particularly useful in providing spatially resolved mass spectrometric analysis (but see also laser desorption). High-energy primary ion beams (typically Cs⁺ or O⁺) typically achieve more aggressive sample sputtering (can be used to ablate surface layers) and yield monoatomic ions suitable for elemental and/or isotopic analysis, whereas low-energy ion beams typically sample only surface layers and yield molecular ions suitable for identification and structural analysis. The former technique is commonly known simply as SIMS (or nanoSIMS, depending on the spatial resolution of the primary ion beam), whereas the latter is often known as TOF-SIMS (although the combination of TOF mass spectrometry with low-energy primary ion beam is not required, it is commonly employed). Note that the acronym SIMS is also commonly used for “selection ion mass spectrometry,” which is a different technique.

Isotope-ratio mass spectrometry (IRMS)—a subcategory of mass spectrometry in which the specific intent is to provide highly precise measurements of isotopic abundance, usually at the expense of losing structural information because analytes must be converted to a common molecular form (*i.e.*, H₂, CO₂, N₂, SO₂, etc.). For organic molecules, such techniques generally employ electron-impact ionization with sector-field spectrometers and multiple parallel detectors. The technique is commonly distinguished from SIMS, even though both provide similar types of information.

Isotope-ratio optical spectroscopy (IROS)—a subcategory of optical spectroscopy in which the specific intent is to provide highly precise measurements of isotope abundance. Specific techniques typically employ either very-long pathlength absorption cells (integrated cavity-output spectroscopy) or cavity-ringdown spectroscopy, and both require that analytes be converted to a common molecular form (*i.e.*, H₂O, CO₂, N₂, etc.). Although the optical detection is non-destructive, conversion to common analyte form is destructive.

X-ray photoelectron spectroscopy (XPS)—a technique where a surface is irradiated with soft x-rays, leading to ionization of the surface atoms. The subsequent release of emitted photoelectrons allows a spectrum to be obtained of the distribution and kinetic energy of the surface atoms to be determined; the intensity of specific peaks allows a quantitative analysis of each analyzed atom.

Processing techniques

Combustion—heating a material in the presence of molecular oxygen, or a source of oxygen, to generate carbon dioxide.

Destructive sampling—sampling or measurement processes, which result in the destruction of the sample.

Solvent extraction—use of a liquid phase to selectively dissolve (solubilize) and separate particular compound classes from a complex matrix. Solvents of different polarities can be used to differentially extract different compound classes.

Pyrolysis—heating a material in the absence of oxygen to induce thermal decomposition. Typically, this approach relies on a defined temperature regimen. Pyrolysis at temperatures up to approximately 600°C is used to convert a solid macromolecular material to smaller, volatile products that were amenable to separation by gas chromatography and identification by mass spectrometric analysis. The composition of these pyrolysis products is used to infer the nature of the macromolecular precursor. Pyrolysis at temperatures exceeding 1000°C typically converts the precursor to its elements (*e.g.*, C, H₂) or small molecules such as CO.

Thin section—a thin slice of sample prepared either for the evaluation of internal composition or to allow access to a technique requiring a thinner cross section of material.

A.3.1. Abbreviations

AC, adventitious carbon.
 ALHT, Apollo lunar hand tools.
 ALSRC, Apollo lunar sample return container.
 AMC, airborne molecular contamination.
 ATLO, assembly, test, and launch operations.
 ATP, adenosine triphosphate, the energy storage molecule of a cell.
 CAPTEM, Curation and Analysis Planning Team for Extraterrestrial Materials, a committee that is part of the NASA advisory structure
 DART/MS, direct analysis in real time–mass spectrometry.
 DNA, deoxyribonucleic acid.
 DOF, degrees of freedom.
 DOP, degrees of probability.
 DRIFT, diffuse reflectance infrared Fourier transform.
 EDAX (or EDX), energy-dispersive spectroscopy.
 EDL, entry, descent, and landing.
 ESA, European Space Agency.
 FTIR, Fourier transform infrared.
 GC-MS, gas chromatography–mass spectrometry.
 GSFC, NASA Goddard Space Flight Center.
 IR, infrared.
 ISO, International Organization for Standardization.
 ITAR, International Traffic in Arms Regulations.
 IUPAC, International Union of Pure and Applied Chemistry.
 JPL, Jet Propulsion Laboratory.
 LC-MS, liquid chromatography–mass spectrometry.
 LM, Lunar Module.
 LRL, Lunar Receiving Laboratory.
 Mars 2020, Mars 2020 Mission.
 MEP, Mars Exploration Program.
 MEPAG, Mars Exploration Program Analysis Group.
 MoE, Meeting of Experts, a process used by the U.S. National Research Council.
 MOMA, Mars organic molecule analyzer (an instrument on ExoMars 2018).
 MS-MS, tandem mass spectrometry.
 MSL, Mars Science Laboratory.
 MSR, Mars sample return.
 MSR SSG II, Mars Sample Return Science Steering Group II.
 NASA, National Aeronautics and Space Administration.
 NRC, National Research Council.
 NRC SSB, National Research Council Space Study Board.

NVR, non-volatile residue.
 OCM, organic check material.
 OCP, Organic Contamination Panel.
 OCSSG, Organic Contamination Science Steering Group.
 OSIRIS-REx, Origins Spectral Interpretation Resource Identification Security—Regolith Explorer.
 PAH, polycyclic aromatic hydrocarbon.
 PCR, polymerase chain reaction.
 PDA, photodiode detector array.
 PLSS, Primary Life Support System.
 PP, planetary protection.
 PTFE, polytetrafluoroethylene.
 QCM, quartz crystal microbalance.
 QE, quantum efficiency.
 RAD, Radiation Assessment Detector (instrument on MSL).
 RGA, Residual Gas Analyzer.
 RNA, ribonucleic acid.
 S/N, signal-to-noise ratio.
 SA/SPAH, Sample Acquisition/Sample Processing and Handling (instrument on MSL).
 SAF, spacecraft assembly facility.
 SAM, Sample Analysis at Mars (an instrument on MSL).
 SDT, Science Definition Team.
 SEM, scanning electron microscopy.
 SIMS, secondary ionization mass spectrometry.
 SMD, Science Mission Directorate.
 SRC, sample return capsule.
 SRF, sample receiving facility.
 TAGSAM, touch-and-go sample acquisition mechanism (instrument on OSIRIS-REx).
 TEGA, thermal and evolved gas analyzer (instrument on Phoenix).
 TOC, total organic carbon.
 TOF, time-of-flight.
 UV, ultraviolet.
 WP, witness plate.
 WSTF, White Sands Test Facility.
 XPS, X-ray photoelectron spectroscopy.

A.4. Appendix 4: Summary of instruments and measurements available as of 2014 for investigating organic molecules in rock and soil samples

A.4.1. Notes regarding detection limits and capability of surface spectroscopic techniques. Challenges exist in defining the detection limits and capability of surface spectroscopic techniques, as they are strongly dependent on instrument design and sample/measurement specifications.

Factors that affect technique sensitivity due to optical design include:

1. *Optical throughput* (laser power, transmission of optics, etc.)
2. *Collection efficiency* (f/#, DOF, DOP, etc.)
3. *Detector sensitivity*
 - a. Noise (dark current, shot noise, read noise etc.)
 - b. Performance (dynamic range, gain, QE, etc.)
4. *Spectral range* (may require time gating to improve sensitivity based on technique)

Example factors that affect technique sensitivity due to sample/measurement specification include:

Key to Measurement Goals Related to Martian Organic Geochemistry and Planetary Protection

1	Determine whether the samples contain organic compounds
1A	Use non-destructive methods to search for the presence of organic compounds
1B	Quantify the bulk organic content of the samples
2	Determine the origin of any organic compounds in the samples
2A	Determine the molecular composition of organics
2B	Determine the isotopic composition of organics
2C	Study spatial variations in abundance and characteristics of organic molecules in the sample matrix, relative to mineralogical, chemical, and textural features
2D	Investigate the chirality of amino acids
2E	Examine long-chain hydrocarbons for chain length effects
2F	Quantify the degree of contamination by viable or recently deceased terrestrial microbes and their residues

SURVEY ANALYTICAL METHODS TO BE USED in LIGHT YELLOW

TARGETED ANALYTICAL METHODS TO BE USED in LIGHT BLUE

Category 1: Non-Destructive, Sample Surface-Based Technique

Analytical Method	Objectives Addressed	Sample Requirements and Degradation ¹	Performance Characteristics and Detection Limits ¹	Method Notes (Dependencies, Limitations, Assumptions, etc.)	References ²
Deep UV Raman/Fluorescence Spectroscopy	1A, 2C	Non-destructive. No surface preparation required.	Raman: Aromatics <10-4 w/w (<100 ppm) Aliphatics <10-4 w/w (<100 ppm) 50 um/spot at 1 to 10s per spot Fluorescence: Aromatics <10-6 w/w (<ppm) Single cell sensitivity (~2 pg carbon) [6] 50 um/spot at 1s per spot	Performance can be enhanced with longer integration times. Sensitivities depend on organic species and are matrix dependent. Surface roughness can be handled based on optical system with hit against sensitivities or integration times. Quantification is difficult	[1] Beegle, et. al., Lunar and Planetary Institute Science Conference Abstracts 45: 2835. [2] Ghosh, et. al, Applied Spectroscopy 66 (9): 1013-21. [3] Tuschel, David D, Aleksandr V Mikhonin, Brian E Lemoff, and Sanford A Asher. 2010. "Deep Ultraviolet Resonance Raman Excitation Enables Explosives Detection." Applied Spectroscopy 64 (4), 425-32. [4] Bhartia, et.al., International Society for Optics and Photonics: 83581A-83581A-9. [5] Johnson, et.al, Astrobiology 11 (2): 151-56. [6] Bhartia et. al, Applied and Environmental Microbiology, 2010, 76(21), p 7231-7237
Confocal Raman Spectroscopy at up to 360nm micron spatial resolution	1A, 2C	Non-destructive. Benefits from thin section, polished surface prep. Or can be fresh fracture surface with contour following confocal optics.	Lower limit from ~0.1 to 1 wt. % per spot analysis (30s) [1] with absolute detection limit correlated to number of analyzed spots. <50 ppm graphitic carbon [1] Single cell detection sensitivity. [2]	Detection limits strongly dependent on laser wavelength, target species. 532 nm excitation provides non-quantitative detection of hematite, beta-carotene. Raman spectra are subject to organic and mineral background fluorescence, which can be mitigated by time-gating. Careful consideration for laser wavelength and power to avoid sample damage. Quantification is difficult	[1] Wang, et. al. Journal of Geophysical Research, 108(E1), 5005 [2] Ref TBD
FT-IR Spectroscopy	1A, 2C	Non-destructive. Benefits from thin section and polished surface prep, but can be used on unprepared surfaces. Ideally KBR pellets are made of samples.	Lower limit ~5 ppm for specific targets 10 um/spot >200 min per spot [1]	Not sensitive to graphitic carbon. Samples are ideally crushed and made into KBR windows [2] Quantification is difficult	[1] Ref TBD [2] General approach for FTIR in literature. [2a] Bhaskar, Nature and Science, 2009;7(5), 45-51 (Dergoan H5 Chondrite) [2b] Matrajt, et. al., Astronomy & Astrophysics, 416(3), 2003, 983-990 (Tagish Lake Meteorite) [3] Anderson, et. al., Review of Scientific Instruments, 76, 034101 (2005)
IR Reflectance Spectroscopy	1A, 2C	Non-destructive.	Lower limit typically ~0.5-1 wt. % per spot analysis, with absolute detection limit correlated to number of analyzed spots.	Sensitive to only specific organic species. Ideal for rapid mineral context. Quantification is difficult	[1] Not used actively for organics detection

Category 2: Slightly Destructive to Sample Surface

Analytical Method	Objectives Addressed	Sample Requirements and Degradation	Performance Characteristics and Detection Limits	Method Notes (Dependencies, Limitations, Assumptions, etc.)	References
Laser desorption-MS	1A, 2A, 2C	Vacuum exposure, polished thin section or fresh fracture surface, laser beam damage	Semi-quantitative, wide range of sensitivities including sub-fmol.	Specific to PAH or other large conjugated systems. No chromatography, so no distinction of isomers or enantiomers.	
Time-of-Flight Secondary Ion Mass Spectroscopy (ToF-SIMS)	1A, 2A, 2B, 2C	Vacuum exposure, polished thin section or fresh fracture surface, ionization damage	Non quantitative, low ppb sensitivity. Very sensitive to surface contamination. Maps organic and inorganic species. For isotopes: ppt sensitivity, 50nm spatial resolution 1-5 per mil isotopic resolution dependent on instrument and isotope.	Provides context of isotopes. C, N, S, D/H	
LAL Assay	2F	Wipe, swap, extraction. Sample exposed to water/solvent, wipe/swab detritus.		Gram-negative microbes only. Insensitive to gram-positive microbes.	
ATP luminometry	2F	Wipe, swap, extraction. Sample exposed to water/solvent, wipe/swab detritus.	Proportional to microbial metabolic activity	Insensitive to spores	
Microbial plating assay	2F	Wipe, swap, extraction. Sample exposed to water/solvent, wipe/swab detritus.	~0.01% maximum sensitivity to abundance of microbial flora		

Category 3: Destructive of Whole Sample

Analytical Method	Objectives Addressed	Sample Requirements and Degradation	Performance Characteristics and Detection Limits	Method Notes (Dependencies, Limitations, Assumptions, etc.)	References
Total inorganic carbon and total organic carbon	1B, weight % abundance of organic carbon	Both non acid and acid digestion used to separate inorganic from organic	~1-10 ppb in 1 ml of gas or about 1E-11 to 1E-12 g of CO ₂ .	Splitting to NPD detectors, nitrogen may be accessible.	
Total inorganic carbon and total organic carbon	1B, weight % abundance of organic carbon	Both non acid and acid digestion used to separate inorganic from organic	~1-10 ppb in 1 ml of gas or about 1E-11 to 1E-12 g of CO ₂ (??)	Probably similar detection limit to above (methanizer w/ flame ionization), depending upon MS capability. Back calculating the sensitivity dependent upon the background, detector noise, kind of tough to say in general. Evolved compounds other than CO ₂ can be detected. Nitrogen can be done at the same time. Need nitrogen perhaps even D/H.	
Microfluidic Capillary Electrophoresis	2A, 2D, 2F		1 to 10 ppb following extraction, derivatization	Process blanks?	
GC/MS FAME using cyanopropyl stationary phase	2A, 2E, 2F		Detection down to below ~ 1 ng per compound	Detection limits are potentially lower if GC does not have significant non-specific absorption, or other issues. Lower detection limits possible by radio GC or LC using radiolabeled derivatizing agent.	
GC/MS using high temperature GC column, and ammonia chemical ionization				Probably similar detection limit to above (methanizer w/ flame ionization), depending upon MS capability. Back calculating the sensitivity dependent upon the background, detector noise, ... kind of tough to say in general. Evolved compounds other than CO ₂	
Tunable Laser Spectroscopy	2B	Destructive via pyrolysis. Typical amount of sample required per analysis: x mg			
Pyrolysis-MS, Pyrolysis-GC-MS		Destructive via pyrolysis. Typical amount of sample required per analysis: x mg		Does not indicate compounds present, only their fragments.	
Liquid extraction and derivatization followed by GC-MS	2A, 2D, 2E, 2F	Extraction, destructive	Detection limits are compound-specific, but as low as ~1 pmol; more like 100pmol for many hydrocarbons. Nominal mass accuracy in typical system.	Can use library mass spectra to suggest compound class. QqQ-MS can target specific compounds, ultrahigh resolution MS can deduce molecular formulae. Can target chirality (e.g. amino acids, amines, etc). Requires authentic standard for definitive identification.	
LC-MS	2A, 2D, 2E, 2F	Sample crushing followed by destructive solvent extraction, possibly hydrolysis, desalting, and more	Detection limits are compound-specific, but typically ~1 fmol 5 ppm to sub ppm mass accuracy possible	QqQ-MS can target specific compounds, ultrahigh resolution MS (e.g. ToF-MS, FT-MS) can deduce molecular formulae. Different ionization modes (ESI, APCI, APPI) can target different functionalities. Targets M+1 parent ion. Can target chirality (e.g. amino acids). nano-LC can improve sensitivity 10-100 fold. Can couple mass and optical (fluorescence, absorbance) detections. Requires authentic standard for definitive identification. Cannot use library spectra.	
high resolution MS (infusion or DART)		Sample crushing followed by destructive solvent extraction, possibly hydrolysis. Minimal other workup required	Semi-quantitative, wide range of sensitivities including sub-fmol, sub ppm mass accuracy possible	Ultrahigh resolution MS (e.g. ToF-MS, FT-MS) can deduce molecular formulae. Different ionization modes (ESI, APCI, APPI) can target different functionalities. Targets M+1 parent ion. DART required minimal preparation and has ~1 mm spot size. No chromatography, so no distinction of isomers or enantiomers.	
liquid ICPMS		destructive; sample oxidized to sulfate	5 nmol dissolved sulfate at 0.15‰ precision; Paris G., Sessions A. L., Subhas A. V. and Adkins J. F. (2013) MC-ICP-MS measurement of ⁸³⁴ S and ⁴³³ S in small amounts of dissolved sulfate. Chemical Geology 345, 1–12.	targets any sulfur in solution as sulfate; can be used for organic compound-class analysis	
combustion EA-IRMS		destructive	25 nmol N, 41 nmol C, both at ±1.0‰ precision; Polissar P. J., Fulton J. M., Junium C. K., Turich C. C. and Freeman K. H. (2009) Measurement of ¹³ C and ¹⁵ N Isotopic Composition on Nanomolar Quantities of C and N. Analytical Chemistry 81, 755–763.	relatively low sensitivity but high precision (0.1 permil)	
pyrolysis EA-IRMS		destructive	1 ug organic H or O	precision of 2-4 permil for H; O??	
Tunable Laser Spectroscopy	2B	Destructive via pyrolysis. Typical amount of sample required per analysis: x mg			
GC-combustion-IRMS	2B	Extraction, destructive	130 pmol CH ₄ at 0.1‰ precision; Merritt D., Hayes J. M. and Marais Des D. J. (1995) Carbon isotopic analysis of atmospheric methane by isotope-ratio-monitoring gas chromatography-mass spectrometry. Journal of Geophysical Research 100, 1317–1326.	Requires excellent separation of compounds and prior identification of structure.	
GC-pyrolysis-IRMS	2B	Extraction, destructive	25 nmol H as heptadecanoic acid at 2.7‰ precision; Hilkert A., Douthitt C., Schluter H. and Brand W. A. (1999) Isotope ratio monitoring GCMS of D/H by high temperature conversion isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 13, 1226–1230.	compound must be GC-amenable	
GC-ICPMS	2B	Extraction, destructive	20 pmol S as dimethylsulfide, at 0.3‰ precision; Amrani A., Sessions A. L. and Adkins J. F. (2009) Compound-Specific ⁶³⁴ S Analysis of Volatile Organics by Coupled GC/Multicollector-ICPMS. Analytical Chemistry 81, 9027–9034.	compound must be GC-amenable	
PCR	2F				
FISH -- Fluorescence imaging of fluorescently tagged compounds	2F			only useful in very specific conditions for terrestrial contaminants	
ELISA	2F			only useful in very specific conditions for terrestrial contaminants	

1. *Measurement duration*: In general, increase integration time for spectroscopic techniques with increase S/N and therefore sensitivity of the technique (assuming S/N is not driven by noise sources, other spectral interferences limitations, etc.).
2. *Spatial mapping requirements*: Instrument design will be driven by ability to map the core over a given spatial area with a specified resolution. This will drive the optical design and sensitivity. In addition, if the measurement duration is limited, resolution or area can be traded against sensitivity/integration time per spot.
3. *Sample working distance*: The optical design can be optimized for any working distance at the expense of sensitivity or instrument size (f/#).
4. *Surface roughness*: Ability for a technique to handle surface roughness will require trades in optical design versus sensitivity or sensitivity to surface only materials (making it less robust to matrix variability).
5. *Matrix affects*: Spectroscopic technique sensitivities are strongly dependent on the matrix, including
 - a. Background interferences such as mineral fluorescence and required time gating to increase organic sensitivity in techniques like Raman.
 - b. Variability of depth of penetration based on mineral matrix type will affect ability to localize "organic detection" to surface only or will limit the optical designs to confocal or surface approaches. This will limit surface roughness robustness for the techniques.
6. *Species type*: Each spectroscopic technique will have species-specific sensitivities due to molecular interactions (*i.e.*, cross sections for Raman spectroscopy) including technique species-specific interference, which can limit detection sensitivities.

These challenges for defining sensitivity of a survey/spectroscopic non-destructive technique led to an analysis approach that will use a series of instruments that can correlate organics and mineralogy and have complementary sensitivities and specificities.

Future work recommendations would include further constraining the processes and sample expectations to solidify instrumentation requirements, including:

- Time for survey measurement, which will be derived by the spatial area and spatial resolution requirements and sensitivity requirement (integration time, DOF, f/#, etc.)
- Making a compilation of potential contaminant species to assess specific detection limits and interferences.

As a point of procedure, a subset of techniques should be used to analyze identical samples to validate instrument performances and characterize sensitivity and specificity to

common species at practical contamination concentrations. This will also help to identify interference levels that inhibit the ability to identify the scientific relevant organics.

Accordingly, and based on instrument capabilities as of the time of writing in 2014 (Table 3 and Appendix 4), the following mass spectrometric survey methods are recognized as being the most specific and sensitive techniques to detect organic contaminants of concern:

- LC-MS in full scan mode can detect a wide range of polar analytes of biological relevance, including amino acids and oligopeptides, nucleobases and oligonucleotides, intact polar lipids, etc. LC-MS is the preferred means to analyze molecules of any size that are not volatile under normal circumstances. Ionization utilizes the evaporating solvent to assist the addition of either positive or negative charges, most commonly via electrospray ionization or atmospheric pressure chemical ionization.
- GC-MS (also full scan mode) can detect a wide range of molecules that are non-polar and volatile to semivolatile under moderate temperatures. Typical analytes are aliphatic and aromatic hydrocarbons, low-molecular-weight lipids, short-chain carboxylic acids and esters, etc.

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